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# SCIENTIFIC MEMOIRS

BY

# MEDICAL OFFICERS OF THE ARMY OF INDIA.

EDITED BY

SIR BENJAMIN SIMPSON, M.D., K.C.I.E.,
SURGEON-GENERAL WITH THE GOVERNMENT OF INDIA.

## PART III.

## 1887.



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# Notes from the Biological Laboratory attached to the Office of the Sanitary Commissioner with the Government of India.

BY

SURGEON-MAJOR D. D. CUNNINGHAM, M.B.,

SPECIAL ASSISTANT TO THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA.

(*With Plate I.*)

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## I.—On the occurrence of peculiar organisms in the blood of Horses and their supposed pathogenic nature. (Plate I, Fig. 1.)[1]

IN the course of 1880, Inspecting Veterinary-Surgeon G. Evans submitted a report on "Surra" as a newly-diagnosed disease very prevalent among horses in the stations of the North-West Frontier, and, according to his observation, constantly associated with the presence of numerous peculiar organisms in the blood. He showed that inoculation of the blood of diseased animals into healthy ones was followed by the development of the disease in the latter and by the appearance of the parasites in the blood. At the same time it appeared that the presence of the parasites in the blood was not necessarily accompanied by the presence of any morbid effects in the case of certain animals, for a dog which had been inoculated with blood from a case of "Surra," and subsequently given by Dr. Evans to the late Dr. Lewis for examination, continued for long after, and when apparently in perfect health, to show an abundance of the organisms intermittently in its blood. According to Dr. Lewis they were very similar to, if not identical with, the flagellated organisms discovered by him in the blood of apparently healthy rats in 1878.

Subsequently Veterinary-Surgeon J. H. Steel, A.V.D., reported on "an Obscure and Fatal Disease among Transport Mules in British Burma," which appeared in 1883 and continued to prevail in the two following years. This disease he regarded as a relapsing fever closely allied to relapsing fever of the human subject, and identical with the "Surra" of Dr. Evans' Report, being, like it, accompanied by the presence of numerous organisms in the blood. Dr. Steel believed these to be Schizomycetes of spirillar type closely allied to *Spirillum obermeyeri* of human relapsing fever. He, like Dr. Evans, found that the disease was communicable from sick to healthy animals by means of inoculations of blood, and that the parasites were then to be found in the blood of the infected ones.

[1] This paper had been written and submitted to the Press before the author had seen Dr. E. Crookshank's paper on the same subject in the Journal of the Royal Microscopical Society—*Ed.*

During the course of 1886 an epidemic prevailed among horses, ponies, and mules in Meerut which, according to Veterinary-Surgeon W. D. Gunn, A.V.D., was not of an anthracoid but of a typhoid nature, the symptoms during life differing slightly from those of anthrax in various respects, amongst others in the fever being of a relapsing in place of a continued character. The *post-mortem* appearances were described as "very similar to those of anthrax," with the exception that there were ulcerations in Peyer's patches. Dr. Gunn cultivated a drop of blood in gelatinised chicken broth and obtained no Bacillus anthracis, but a crop of minute Bacilli and numerous small micrococcoid bodies which he believed to be spores. He does not appear to have observed the presence of any spirilloid organisms in the blood.

A difference in opinion prevailed among the veterinary authorities as to the nature of the disease, some agreeing with Dr. Gunn as to the specific distinctness of the disease, others holding that "both in its course and *post-mortem* results it resembled ordinary anthrax." Specimens of blood obtained from diseased animals were forwarded to Dr. Barclay for examination with a view to aiding a decision of the question, and some of these were sent on by him to me. The first samples which reached me consisted of two capillary tubes containing blood and closed at either end with common sealing-wax. The tubes were first carefully washed with a solution of corrosive sublimate and with spirit, and were then allowed to dry. The tip of one of them was broken off by means of a freshly-heated pair of forceps and the minute clot and serum introduced into a little freshly-sterilised salt solution of a strength of 0·75 per cent. A portion of the clot was used for preparations and the rest of it was broken up in the solution and used to inoculate a plate-culture of agar-agar peptonised meat-juice. The contents of the second tube were used for preparations. The preparations showed the presence of a few small straight Bacilli and Micrococci, and, in one instance, numerous large spirilloid bodies at a particular part of the clot. The cultivation yielded a few colonies of straight Bacilli of considerable size and innumerable colonies of micrococcus luteus. Subcutaneous injection of a solution full of the straight Bacilli into the bodies of guinea-pigs produced no appreciable result.

The second set of samples consisted of four of the common microscopical glass slides with a central hollow on one surface. The hollows contained blood and were covered by cover-glasses. The contents were used for plate-cultures and preparations. In one specimen the clot was full of small straight Bacilli, in the others the characters agreed generally with those of the previous samples, but in none of them could any spirilloid bodies be detected. The results of cultivations were similar to those of the cultivation from the first sample, and in no case was there any evidence of the presence of Bacillus anthracis.

The spirilloid organisms in one of the first set of preparations presented the following characters. They were present in large numbers in the thin stratum

of blood along a portion of the edge of a thick mass of clot among the blood corpuscles which had been pressed out of it in the course of preparation. They consisted of filaments of various lengths, some merely curved or sinuous, others, and specially the longer ones, showing one or two distinct spirals. Both extremities appeared to be pointed, but one was apparently thicker and somewhat blunter than the other. In some cases a slender flagellar process could be recognised continued for some distance from the more pointed extremity. The length of the larger specimens omitting the curves was about 25 $\mu$, the breadth varying in different parts of the thicker portions of the body from 1·5 $\mu$ to 0·78 $\mu$. The filaments were sometimes only very slightly curved, but were in no instances straight. They were always quite smooth in outline and showed no granularity of texture. In one specimen a pale vacuole-like area was present in the middle of the length of the filament. There can, I think, be little doubt that these bodies are identical with those described by Drs. Evans and Steel. The fact that only mounted specimens came under observation rendered it impossible to ascertain the character of their behaviour in the blood, but their general structural features agree very closely with those described by Drs. Evans and Steel as present in their specimens, and, as Dr. Lewis pointed out in regard to the latter, are almost identical with those of the organisms discovered by him in the blood of rats. The measurements given by Dr. Lewis of the organisms in the rats' blood are 25 $\mu$ by 0·8 $\mu$ to 1 $\mu$, practically identical with those of the larger filaments in the present instance.

The above facts clearly go to show one thing, namely, that the term anthrax has been used very loosely as a descriptive term for various forms of disease in horses in India, and that some of these are certainly not specific anthrax dependent on the invasion of the system by Bacillus anthracis. It is only now when systematic thermometrical observations are carried out and cultivations of the blood are conducted that this begins to be appreciable, and it certainly is very desirable that the matter should be thoroughly investigated and trustworthy information obtained to what extent true anthrax really does occur. It certainly is remarkable that with such extremely common occurrence of so-called anthrax in horses so little is heard of the disease in cattle.

It also appears that in certain forms of equine disease peculiar spirilloid organisms occur in large numbers in the blood, and that the disease has occurred in healthy animals subsequent to inoculation of such blood, but this is all which has been as yet demonstrated. The statement that the disease is closely related to human relapsing fever is certainly not supported by the thermometrical data, and the truly spirillar nature of the parasitic bodies has yet to be demonstrated. Even were this conclusively done, there would still remain an absence of evidence to show that they are causally related to the disease. There is apparently as yet no evidence that it has been definitely ascertained that such organisms are never present in horses in health, as they

were in the case of the dog into which they were introduced by inoculation, and as, at all events, similar organisms have been shown by Dr. Lewis to occur in the blood of healthy rats. It has further to be shown that they are invariably present in cases of the disease. In so far as thermometrical data go, the disease observed by Dr. Gunn at Meerut appears to have been identical with that studied by Dr. Steel in Burma, and yet the presence of such organisms in the blood was not apparently observed, and in so far as my own observations go was not constant. This may, no doubt, possibly be due to the specimens of blood having been taken at times when the organisms were absent or present in very small numbers in the general circulation, as it is recorded that their appearance is of an intermittent character, and, as regards the samples examined by me, may possibly have been connected with the length of time elapsing between the period at which they were taken and that at which they came into my possession. The facts are, however, at least such as to render it additionally necessary that definite evidence should be forthcoming to show the constant association of the disease with the presence of the parasites. The fact of the occurrence of the disease in previously healthy animals and the appearance of the parasite in their blood after inoculation do not afford data of any conclusive value. They certainly show that the parasites can be transferred from the body of one animal to that of another, but that is all. There is nothing to show that they gave rise to the associated disease. No clean artificial cultivations have as yet been carried out, and all inoculations have been conducted by means of blood directly transferred from the diseased to the healthy animal; so that, fully granting that the disease has been shown to be inoculable and that the parasites are similarly transferable, there is yet nothing to show that the two phenomena hold any beyond a parallel relation to one another; there is nothing to show that the transplantation of the parasites was the cause of the development of the disease in the inoculated animals. Whilst, on the one hand, there is this entire absence of positive evidence of the pathogenic function of the parasites, there is, on the other hand, some evidence pointing in an opposite direction—evidence which is, at all events, calculated to raise additional doubts as to their essential relation to the disease. This lies in Dr. Lewis' observations, which have been already referred to, on the persistent intermittent occurrence of large numbers of the organisms in the blood of a dog long after inoculation and quite apart from the presence of any morbid symptoms.

## II.—On a peculiar, truly spirillar form of the Choleraic Comma-Bacillus. (Plate I, Fig. 2.)

Truly spirillar forms of the choleraic comma-bacillus appear to occur with extreme rarity in cultivations in Calcutta. It is, of course, far from uncommon to meet with cultivations in which many of the elements are associated so as to form wavy filaments of various lengths. These are, however, as a rule,

not truly spirillar, the constituent elements really lying in one plane and the filaments having no tendency to assume a spiral arrangement, but consisting merely of a series of commas adhering to one another by their extremities in simple linear order. The character of such filaments varies according as the constituent commas present their convex and concave outlines throughout to the same sides of the filament, or as they are disposed, so that an alternation in the direction of their outlines is present, the convex and concave contours of adjacent commas being directed to opposite sides. The outline of the filaments in the first case presents a series of crescents with intervening depressions and prominent points running along the one side, and rounded elevations and angular inter-spaces on the other. In the second case the filament is alike on both sides and simply undulate. Whatever be the variation in detail, however, such filaments agree in their non-spiral character.

So rarely does a truly spiral arrangement appear to be that I have only on one occasion met with unequivocal evidence of its presence. This was in the cultivation of materials which were derived from a case of cholera which was admitted into the Medical College Hospital, Calcutta, in the month of January. A primary plate-cultivation from it furnished no colonies of typical commas alone, but many which, while containing a certain proportion of isolated commas, were composed in greater part of peculiar filaments, many of which at one or other point in their course assumed a truly spiral character. Others were merely undulate and many S-shaped bodies were also present. In those specimens in which true spirals were present the filaments throughout the greater part of their length were merely undulate and in some cases almost straight, and it was only here and there at isolated points that abrupt spirals of from one to three turns were present. The numbers of such filaments were very large as compared with those of the other forms present in the colonies. In certain filaments, in place of actual spirals, sudden deep curvatures were present, the curved portions being considerably thicker than the rest of the filaments and closely resembling the larger varieties of curved Schizomycetes occurring so abundantly in the intestinal contents of healthy guinea-pigs. The protoplasmic contents of the curved portions were often distinctly vacuolate. Attempts at the continued propagation of this peculiar variety of choleraic commas failed, as even in secondary cultivations true spirals were entirely absent and filamentous forms of any kind rare, while in tertiary ones the colonies consisted of typical commas alone.

## III.—On the occurrence of Schizomycetes in the peritoneal secretion of cases of Cholera.

After it had been ascertained that in certain cases of subcutaneous injection of choleraic comma-bacilli in guinea-pigs a very excessive multiplication of

them occurred within the peritoneal cavity, numerous examinations and cultivations of the peculiar adhesive peritoneal secretion in cases of cholera were made. In no instance were Schizomycetes of any form recognizable in any considerable numbers in the fresh materials. In some cases a very few long, straight filaments were present, in some a few patches of Micrococci occurred, and in one case, at all events, minute groups of small curved Bacilli, resembling choleraic commas, were present in small numbers. In no instance did choleraic commas appear in the cultivations, but in one a distinct and peculiar curved Schizomycete form was developed. The commas were larger and thicker than choleraic ones; they did not liquefy the gelatine of the basis, but slowly formed thick, prominent, shining growths of a pale salmon colour. When subcutaneously injected into the bodies of guinea-pigs they produced no appreciable effects.

## IV.—Results of examinations of old specimens of Choleraic tissues for "the Cambridge Cholera Fungus."

After the publication of the "Preliminary Report on the Pathology of Cholera Asiatica (as observed in Spain in 1885)" by Messrs. Roy, Brown, and Sherrington in the Proceedings of the Royal Society,[1] and the discussion to which it gave rise in the pages of *Nature*,[2] a careful examination was made in order to ascertain whether any organisms, such as those described and figured in the report, could be detected in choleraic tissues in Calcutta. A considerable amount of time was devoted to the matter, and a very large number of preparations were made, as, quite apart from any special choleraic interest, it appeared to be well worth while to expend time and trouble in the attempt to obtain specimens of organisms of such unique characters as apparently to resemble indifferently members of the Family Chytridiaceæ and Bacteria in "an involution form." The materials examined, like those in which the organisms are described as occurring, were derived from cases of cholera which occurred in 1885 and had been continuously preserved in absolute alcohol from the time at which the *post-mortem* examinations were made shortly after death. They consisted of small portions of the duodenum, jejunum, ileum, cæcum, liver, spleen, kidney, and mesenteric glands. Numerous sections were cut from the specimens belonging to six different cases, and various stains were employed, special attention being naturally given to staining with methylene blue, particularly after Löffler's method, but in no single instance could a trace of any organisms resembling "the Cambridge Cholera Fungus" be detected. The examinations further showed anew how exceptional it is in Calcutta to meet with cases in which there is any penetration of the intestinal tissues by the

[1] Vol. XLI, No. 247, p. 173. [2] Vol. 35, pp. 171, 271, 295, 319.

choleraic commas, for in not a single case was there any evidence of the presence of any such phenomenon.

### V.—On Fungal contamination of the Gelatine and Agar-Agar in sterilised tubes during the Rains in Calcutta.

On returning to Calcutta in 1886, after an absence of more than two months during the rainy season, I found that almost all the sterilised tubes of common gelatine and agar-agar which I have left in the laboratory showed manifest signs of contamination, the surfaces of the nutritive media being covered with conspicuous white growths. As many of these tubes had remained for months previous to my departure in the end of the hot weather without showing a trace of any contamination, it appeared at first sight difficult to account for the phenomenon. Microscopical examination of the nature of the growths present in the tubes appeared, however, to afford a plausible explanation of it. It was ascertained that in no case did the growth consist of Schizomycetes, but that they were composed of filamentous mould-fungi. Now, the conidia of such bodies are very much more abundant in the air in Calcutta during the rains than at other times, and the general moisture of the surfaces on which they may be deposited favours their germination. The infected tubes were closed by two superimposed plugs of sterilised cotton wool, but were not capped, so that particles of atmospheric dust were free to be deposited on the upper surfaces of the superior plugs.

During the cold and hot weather, the number of conidial elements thus deposited was relatively small and the cotton wool was too dry to favour their germination, and at this time contamination did not occur. With the rains, however, new conditions were established : the numbers of fungal elements liable to deposition became greatly increased and the cotton wool became gradually moist enough to allow of those which were deposited germinating and to form a medium in which continuous mycelial filaments could readily spread. Gradual penetration of the entire thickness of the plugs by mycelium was therefore liable to occur, and when this had been established, any free conidial elements arising on the under surface of the inferior plug would of course be very likely to be deposited on the surface of the nutritive medium in the lower part of the tube. The plugs remained effective obstacles to the access of Schizomycetes, because they did not present suitable nutritive materials to allow of growth of Schizomycetes in such quantities as to penetrate the thick obstructive strata. The higher fungi were at an advantage because of the greater amount of store-nutritive materials which their ordinary reproductive elements contain, and because of their mode of growth which permits of the continuous progress of the protoplasm from one area to another, and therefore render it possible that the comparatively small amounts of nutritive materials present at different points should be successively utilised in assimilation.

## VI.—On a destructive blight in Tea plants dependent on the invasion of the tissues of the roots by the mycelium of a fungus.

During the cold weather of the current year I was asked by Dr. King, the Superintendent of the Royal Botanical Garden, Calcutta, to examine some specimens of root-blight in tea which had been submitted to him for an opinion as to the nature of the disease. A thoroughly decisive determination of certain points in connection with the disease could only be attained by means of experiments on infection of living plants which I have had no opportunity of attempting in Calcutta, and others certainly call for more extended observation than it has yet been possible to give to them ; still, as the information at present available appears to be of some economic value, it has seemed better to put it on record at once than to wait until the observations are theoretically perfected.

The specimens, as I obtained them, consisted of the lower parts of the stems and the attached larger roots of the plants. The latter and the basal, and probably subterranean, portion of the stems showed conspicuous malformations. At intervals along the course of the roots there were a series of rough swellings where the bark no longer retained its smooth surface and grey colour, but was evidently broken up and mixed with masses of soil. Towards the origin of the roots the swelling became continuous and was very great, extending thence over the lower end of the stems and thence gradually thinning off and disappearing a few inches above. The texture of the swellings was friable, and they evidently consisted of a mixture of dead disintegrating masses of bark tissue, mixed with earth in varying proportions. On examining them closely they presented a variegated appearance, due to the deep brown of the dead bark tissues mingled with the brownish-grey soil. Here and there, too, masses of a yellowish or rusty colour could be seen projecting from the general surface, and on detaching portions of the surrounding material these were found to be continuous with sheets and strands of similar colour diffused throughout the looser superficial portions of the swelling. Similarly coloured areas were also present in the deeper, denser parts of the bark and cambium, and, immediately over the external surface of the wood, they were spread out so as in many places to form an almost continuous thin layer. The colour here was in some places much paler than more superficially, in certain cases being almost white. On transverse section the wood was found to retain its hardness unaltered and in most places appeared to be entirely unaffected. Here and there, however, there were more or less wedge-shaped areas of grey discolouration, with narrow, sinuous blackish margins separating them from the surrounding normal pale yellow wood.

On detaching portions of the rusty material and putting them into water

they were found to present a spongy texture, and on teasing them out under a dissecting microscope they were manifestly composed of fibrous elements. When such teased material was subjected to higher microscopical powers, it was evident that it was composed of fungal mycelium, consisting of long, flattened filaments of very various diameters and of a colour ranging from strong yellowish-brown to pale greenish. They were septate, the septa in most places occurring at wide intervals, but here and there being comparatively close set. Branches were given off at varying, but always relatively remote, intervals, and there was frequently a slight swelling of the filament immediately beneath the point of origin of a branch. The filaments in the larger masses formed dense, felted masses, intermingled with portions of brown dead bark-tissue and particles of earth, and continuous with finer strands which penetrated the surrounding areas. On making sections of portions of bark in which the disease had not advanced far and in which the tissue still remained coherent, the bark and cambium down to the level of the wood was found to contain fine mycelial filaments forcing their way between the adjacent tissue elements. In these cases there was no evidence of any invasion of the wood at this period, but from the microscopic appearances presented by the latter in certain places in sites where the disease was more advanced there can be no doubt that such invasion eventually occurs.

Numerous attempts have been made to cultivate the mycelium in order to determine the nature of the fungus, but hitherto the results have not been such as to yield conclusive data. Portions of the roots when kept in moist chambers invariably soon showed a dense coating of a vivid green mould over the diseased areas, but it has not appeared definitely whether any organic connection exists between this and the original mycelium. It certainly was not a form native to the laboratory in which the cultivations were conducted, but unequivocal evidence of direct continuity of the brown mycelium with the colourless filaments giving origin to the fertile threads of the mould has not yet been obtained. The mycelium of the mould is colourless and septate at frequent intervals. It gives origin to numerous fertile hyphæ. These also are colourless and septate, and either give off secondary axes or cells directly bearing heads of fructification. The latter consist of a series of short cells bearing from one to four sterigmata giving origin to chains of rounded conidia. The conspicuous colour of the mould is due to the latter, as, even when isolated, they have a distinct greenish tinge, and when in masses appear of a deep vivid green. They vary considerably in size, but the larger ones on an average have diameters of $4{\cdot}1\ \mu$ by $3{\cdot}5\ \mu$. The heads of conidia form dense rounded masses, at first sight simulating heads of aspergillus, but the minute structure is always as described above. The masses of fructification appear at first as isolated prominent green cushions on the surface of the diseased tissue, but as they extend they in many places become confluent and form a continuous green coating. One other form of

mould, a small pink Stilbum, and an inconspicuous mucorine fungus, also appeared on some specimens of the bark, but neither with the same constancy nor in such profusion as the mould described above. In no case was there any attempt at the development of any Hymenomycete form of fructification.

In some cases a distinct growth of the rusty mycelium occurred during the course of cultivation. The newly developed portions were characterised by their frequent septation and by giving off numerous erect filaments densely clothed with short branches tipped with numerous radiating processes. These suggested sterigmata, but in no case could any development of conidia from them be detected. Those portions of the mycelium where such growth occurred came to present a dusty, bright orange-coloured surface due to the tufts of branched filaments.

Owing to the failure of the cultivations in affording conclusive results in the form of fructification, it is as yet impossible to identify the nature of the mycelium producing such destructive effects on the bark tissues, and without experiments on artificial infection it is even uncertain whether the disease be really essentially of fungal origin or whether the presence of the fungal elements is due to antecedent morbid conditions in the bark. There can be no doubt as to the destructive effect of the mycelium on the host tissues; the only doubt is whether the mycelium is capable of attacking perfectly healthy tissues. That it can, and that it is therefore the essential primary cause of the disease, is rendered highly probable by the general similarity in the distribution and characters of the morbid changes to those present in the case of the root-blight which is so destructive in European coniferous forests and which has unequivocally been proved to be due to the invasion of the tissues by the mycelium of *Agaricus melleus* (Vahl.). It does not, however, appear probable that the latter is the destructive agent here, for the mycelium in the tea-blight does not show any tendency to assume a truly Rhizomorphic habit as that of *Agaricus melleus* does, and if the mould appearing in the cultivations really hold an organic relation to the mycelium, the latter cannot, of course, belong to any Hymenomycete, but must be of Pyrenomycete nature. These are questions, however, which call for further investigation, and all that can be safely stated at present is that in this blight we have to deal with a disease in which fungal mycelium plays an important, if not a primarily essential part, and that it is possible that an important agency in securing the diffusion of the disease lies in the conidia developed in such abundance on the morbid surfaces when these are exposed to damp air.

Even with the confessedly imperfect data at present available, there can be no doubt as to the practical measures which should be adopted with a view to preventing the spread of the disease. All blighted plants should be at once and completely removed. The ground where they occur should be carefully and deeply dug over, and all portions of the roots as far as possible removed

and, together with the other parts of the plants, at once burned. An area in which the blight has appeared should be left unoccupied for some time, and, if possible, should be isolated by means of encircling it with a trench excavated to a level below that to which the roots of the plants descend. By means of such measures a limitation to the spread of the disease may be looked for, but without isolation, and specially without careful removal and destruction of all portions of morbid tissue, the disease, once established, may be expected to go on continuously radiating over areas of wider and wider radius.

## On the Results of Cultivations of Choleraic Comma-Bacilli in Earth, in Cowdung, and in Human Excreta.

One of the great objections to accepting the view that the choleraic comma-bacilli are really the essential cause of the disease is their apparent incapacity for assuming a resting condition and their apparent general feebleness in the struggle for existence. There were many phenomena on record regarding the occurrence of outbreaks of cholera within areas where the disease is only an occasional epidemic visitor which certainly appear to indicate that, if the morbific agent be of an organised nature, the causative organism must be capable of enormous multiplication under exposure to the prevailing local conditions at certain times, and that at others it must be able to pass for prolonged periods into a condition of dormancy. Within the endemic area, too, certain phenomena in the seasonal cycle of prevalence are very hard to explain, save as dependent on the alternation of periods of activity and of rest, or, at all events, of periods during which the local conditions favour or repress the multiplication of the exciting cause. During the course of the past season, a renewed attempt has been made to determine how far the choleraic comma-bacilli are capable of holding their own under external conditions such as those prevailing in Calcutta, and in mediá to which they are specially likely to gain access in large numbers under ordinary circumstances. The media to which choleraic materials are specially likely to attain access in bulk are, of course, water and soil of varying degrees of purity or organic contamination, and as it appears to have been clearly shown that the commas rapidly disappear from water without any attempt at the development of resting forms, attention was specially directed to the subject of their behaviour in soil. A series of experiments was, therefore, initiated in the middle of December, that is, just at the time at which the minor of the two annual periods of minimum prevalence of cholera in Calcutta normally begins to manifest itself, on the effects following the introduction of large masses of choleraic commas into portions of common garden soil.

On the 16th of December two small glass beakers were filled with garden-earth which had been thoroughly triturated with a certain amount of fresh, normal human excreta. The contents of five tube cultivations of cholera commas

were then added to them, each receiving the contents of one and a half tubes of common gelatine and one tube of agar-agar gelatine mixed with a certain amount of freshly-sterilised salt solution and full of healthy commas. They were then set aside under a bell-glass on a table in the laboratory. Both beakers were allowed to remain thus until the 6th of January 1887. At this time the surface of the soil in each was still moist and showed scattered patches of greyish gelatinous matter, seemingly remains of the gelatine of the cultivations, together with a few heads of *Pilobolus crystallinus*. One of them was now set on the incubator to dry, and materials for cultivation were removed from the other. These consisted of portions of the gelatinous matter from the surface. Some of these were broken up in sterilised salt solution and used to start plate-cultivations, while others afforded material for preparations. The material was faintly alkaline. It contained no active Schizomycetes, but numerous fungal cells and encysted fæcal amœbæ, and innumerable still Schizomycete forms, especially in the spore-condition. The plate-cultivations yielded an abundant crop of colonies of various kinds of Schizomycetes, but, in spite of careful examination, no commas could be detected.

On the 18th January another preparation and a plate-cultivation were made from the same sample of earth. On this occasion the materials were taken from about a quarter of an inch beneath the surface. No comma-bacilli were recognisable in the preparation. The plate-cultivation yielded a very abundant crop of Schizomycete colonies after it had been for twelve hours in the incubator. It had a peculiar, strong, bat-like odour, very unlike that characteristic of cultivations of the choleraic comma-bacilli. Two distinct forms of Bacilli were present in large numbers, but not a trace of commas could be detected anywhere.

On the 1st March another plate-cultivation from the same soil was initiated. The soil was now fairly dry, and after the material required for cultivation had been removed, some freshly-boiled tap water was poured over it. The cultivation yielded numerous Schizomycete colonies. They all consisted of straight Bacilli of various sizes, and no signs of commas could be detected anywhere. Subsequent cultivations of the same sample of earth also failed to yield any commas.

On the 18th March the beaker which had been in the incubator since the 6th January was examined. The earth was found to be perfectly dry. A small portion was removed and mixed with freshly-sterilised salt solution to form the basis of a plate-cultivation, a large quantity of freshly-boiled distilled water was then poured into the beaker, so as to flood the soil, and the beaker was replaced in the incubator. On the following day the plate-cultivation showed numerous colonies, consisting of two forms of straight Bacilli, but no commas could be found. On the 21st March the surface water had disappeared from the soil in the beaker. Materials for a plate-cultivation were removed, and the earth was again flooded with freshly-boiled distilled water. On the following day the plate-

cultivation had developed large numbers of yellowish, almost odourless colonies of considerable size, consisting of straight Bacilli. No commas ever appeared in it. Subsequent cultivations from this sample of soil also failed to produce any commas.

In the above experiments we find that very large quantities of comma-bacilli introduced into fæcally contaminated soil and exposed to conditions similar to those to which the Bacilli entering the soil in Calcutta are normally liable during the period of year dealt with, failed to multiply, and, on the contrary, rapidly and completely disappeared. In dealing with any case in which the possibility of the existence of resting forms is present, it is, of course, necessary to exercise caution in concluding that an organism has finally disappeared because it fails to manifest its presence under conditions in which the active form flourishes. The resting stage by no means necessarily is replaced by the active one on the mere incidence of conditions favourable to the latter and at certain times certainly serving to bring the resting stage to a close. The element of time is often an important factor in the result. This is very conspicuously evident in the case of many teleutospores of uredinal fungi, and also in the resting cells of some of the unicellular algæ. In the present case, however, it may, I believe, be safely assumed that the phenomenon of failure of appearance corresponded with actual death of the commas, because the period of observation was sufficiently prolonged to extend beyond that at which, if the phenomenon of temporary subsidence of the prevalence of cholera in the cold weather be so interpreted, there is a tendency to the assumption of a resting condition on the part of the Schizomycetes which are related to it.

Allowing that the experiments seemed to show that the choleraic commas rapidly and completely died out in these portions of soil, the possibility remained that this result was due, not to any incapacity on their part for continued life in soil apart from any adverse agencies represented by other organisms, but was due to the presence of the Schizomycetes and Fungi of the fæcal matter with which the soil was contaminated. This point was so far, at all events, settled by means of the following experiment, which was carried out sumultaneously with those which have just been considered. In it a beaker was filled with common clean garden-earth, and a considerable quantity of a fresh choleraic evacuation was then poured over the latter, so as to saturate it thoroughly. This was done on the 17th December 1886, and the beaker was then set aside beneath a bell-glass. The earth gradually dried up and soon was apparently nearly dry. On the 26th January 1887 a plate-cultivation was set of materials derived from it. An extremely abundant development of Schizomycete colonies of various kinds resulted, but no commas were to be found among them. On the 23rd March the earth appeared to be thoroughly dry throughout, and some freshly-boiled distilled water was poured over it, so as to flood it and form a surface layer of standing water. The earth was now

thoroughly stirred up with a sterilised platinum wire and a plate-cultivation set from it. A limited number of colonies became developed in the cultivation, but none of them contained choleraic commas. The majority of the colonies consisted of straight Bacilli, and a few of them of peculiar, thick, slightly curved ones. Repeated cultivations of these were made, lest they might be a modified form of the choleraic comma-bacillus, but the result was to show that they were permanently and specifically distinct. Another plate-cultivation of the earth was set on the 29th March. Numerous colonies, all composed of large straight Bacilli, were developed in it, but, as before, no commas appeared. The above experiments having clearly shown that choleraic comma-bacilli rapidly and permanently disappear from portions of soil, whether pure or fæcally contaminated, exposed to the ordinary conditions prevalent in Calcutta, an attempt was made to ascertain whether any other materials to which they are likely to gain access were more favourable to their continued existence. The material selected for experiment in the first place was cowdung, which, from the extent to which it enters into the domestic economy of the lower classes of natives, is constantly at hand to receive choleraic contamination. Some fresh cowdung was procured on the 23rd of March 1887, and two separate portions of it were inoculated with masses of agar-agar full of choleraic commas. In both cases the portions of dung were set in sterilised watch-glasses in moist chambers, but while the dung in one case was set in its natural condition, in the other it had been previously mixed with distilled water and subjected to prolonged boiling. Five days later small portions were removed from each, at sites far removed from the sites of inoculation, and were used to start plate-cultivations. In both cultivations a great number of Schizomycete colonies were developed. In that corresponding to the boiled dung these, in so far as could be ascertained, all consisted of vigorous, well-developed choleraic commas. In the cultivation corresponding to the unboiled dung commas were also present. They were not, however, apparently so vigorous, but were slender and stained feebly, and they were accompanied by numerous colonies of large, straight, Bacilli. The boiled dung appeared, therefore, to represent a pure cultivation of vigorous choleraic commas, the unboiled dung a mixed cultivation of comparatively feeble commas and of other Bacilli. The boiled dung was now removed from the moist chamber and was kept in the incubator for 72 hours at a temperature of 37°C. At the close of this period it was quite dry throughout and readily broke up into dust on being disintegrated. A plate cultivation was inoculated from some sterilised salt solution in which portions of the dried material had been allowed to soak for some time in a state of fine division. An abundance of colonies became developed in it, a few consisting of Micrococci, and the great majority of straight Bacilli. Not a trace of the presence of any commas could anywhere be detected in it.

A second portion of fresh cowdung was procured on the 30th March.

A mass of it was set in a sterilised watch-glass in a moist chamber, and a plate-cultivation was started at the same time. Numerous colonies appeared in the latter. They were of small size and yellowish colour, and were accompanied by a faint odour quite distinct from that present in cultivations of choleraic commas. They all appeared to be alike and composed of very short, thick, almost micrococcoid Bacilli. On the 31st March the cowdung was inoculated with a mass of agar-agar full of commas. Numerous plate-cultivations were carried out from time to time up to the 20th April, but in no case did any commas make their appearance. Numerous colonies developed in all cases, but these never contained commas. The majority of them consisted of short, thick Bacilli, which, when in mass, had a peculiar greenish-yellow colour, and which imparted an odour to the cultivations very similar to that of cultivation of commas. Repeated cultivations showed clearly that they bred perfectly true under conditions most favourable to the development of commas, and that they, therefore, were specifically distinct from the latter. On the 20th April a plate-cultivation was inoculated from the portion of agar-agar introduced into the dung on the 31st March, and which was now, curiously enough, absolutely crowded with the myceleum and zygospores, or, perhaps more correctly, oospores, of a small mucorine fungus, apparently parasitic on *Pilobolus crystallinus*, which at this comparatively late period had begun to appear in abundance on the surface. There were no recognisable commas in the agar-agar, and the cultivation failed to produce anything save a few minute colonies of straight Bacilli.

The above data appear to indicate that cowdung, apart from the organisms normally present in it and destroyed by boiling, constitutes a highly favourable medium for the development of choleraic commas, but that in its normal state the development is either entirely repressed or very much enfeebled, due to the influence exerted by other fungal and Schizomycete organisms. They also clearly show that in this medium also the commas have no capacity for the assumption of a resting condition.

The first set of experiments had appeared to show that human excreta did not constitute a favourable medium for the growth of choleraic comma-bacilli, but it appeared desirable to endeavour to ascertain how far the results then attained were not due to the acid fermentation normally occurring in such materials in connection with the abundant development of fungi usually occurring in them during the earlier stages of decomposition. Some normal, fresh, almost neutral human fæces were accordingly diluted with distilled water and strongly boiled for some time, and a portion of the material was then set in a sterilised watch-glass in a moist chamber. On the following day the re-action was distinctly and permanently alkaline. A portion of agar-agar, full of healthy choleraic commas, was now introduced. Forty-eight hours later the material had a peculiar mawkish smell and was alkaline in re-action. From this time

onwards repeated plate-cultivations were inoculated from it, but even when the inoculated materials included portions of the agar-agar, they yielded no commas, although producing an abundance of various other forms of Schizomycetes. It would appear from this that human fæces are even less favourable to the growth of commas than cowdung, the temporary repression in the growth of other organisms consequent on boiling of the medium failing to produce the effect that it does in the case of cowdung. All the data seem to indicate that the choleraic comma-bacilli are, under ordinary circumstances extremely feeble in the struggle for existence. In media, such as fresh cowdung appears to be, which in themselves afford everything necessary to their continued vitality and multiplication, the presence of other organisms normally present in the medium is sufficient to repress or absolutely suppress further development, and in other media, such as human fæcal matter, even the aid of artificial measures calculated to place them at an advantage in the struggle are insufficient to be of any avail in securing their continued existence. Not only are they weak in the active struggle for existence however, but they are also apparently incapable of tiding over periods during which the media are in any way unfavourable by assuming a resting state. It is, no doubt, true that in many sterilised media in which they are not exposed to competition with other organisms they are capable of enormous and rapid multiplication, but under ordinary circumstances they do not gain access to sterilised media, but to media already occupied by their normal inhabitants. If the choleraic commas really be the essential cause of the disease, then the facts would lead us to regard their agency in the production of epidemics as entirely subordinate to the influence of local conditions securing the presence of media of peculiar quality favouring their continued development. Normal water, soil, and excreta, or the normal media to which choleraic commas are specially liable to gain access, do not seem to provide against their speedy extinction, so that it can only be where some very special medium is present, or where the common media deviate from their normal standard, that there can be sufficient provision to secure the multiplication and continued persistence of the commas to such an extent as to accord with many of the phenomena of epidemic diffusion of the disease.

CALCUTTA,
*The 15th May 1887.*

# Note regarding certain characters in the Subsoil of Calcutta.

BY

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The scheme for the construction of a dock at Kidderpore, one of the suburbs of Calcutta, has afforded special facilities for acquiring information, both in regard to the nature of the subsoil and the extraordinarily incorrect ideas which are commonly entertained regarding it. When the question of the construction of the dock was still under consideration, it was authoritatively announced that the subsoil in which it was intended to lay the foundations consisted of "blue clay," that it contained no subsoil water, and that its impermeability almost equalled that of cast-iron, so that no accumulation of water would ever occur in the excavations necessary for the construction of the dock beyond that due to direct rainfall so long as means were taken to exclude surface drainage. Having for many years had special occasion to consider the nature of the subsoil in Calcutta, I entertained great doubts as to the correctness of these statements, and accordingly tried a series of experiments with the view of obtaining actual data in place of "speculative opinions," as the advocates of the dock-scheme styled all views which tended to cast any doubt on the impermeable nature of the soil. The present note contains an outline of these experiments, the result of which was to show clearly that the so-called "blue clay" is not clay at all, and that, in place of being impermeable, it contains a very high proportion of air or water content according to the conditions to which it is exposed.

The materials experimented with consisted of samples of the "blue clay," one obtained from a member of the Committee which was appointed to enquire into the sanitary aspects of the dock-scheme, the other procured by myself from the bottom of the initial excavations for the dock-walls. Both were precisely alike, consisting when first obtained of a slate-coloured, rather evil-odoured, dense, moist substance, permeated in many places by channels partly or wholly filled with soft brownish matter and corresponding with the sites at one time occupied by the smaller roots of trees, which formerly grew on the present subsoil, and whose remains occur in abundance in it. On keeping the material, it rapidly parted with its moisture, becoming, as it did so, of a pale grey colour and hard consistence. Specimens were submitted to analysis

both in the laboratory of the Assay Department of the Calcutta Mint and in that of the Chemical Examiner to Government with the following results:—

(1) Analysis in the laboratory of the Assay Department, conducted by elutriation on samples which had been submitted to prolonged drying at 100°C.—

| | |
|---|---|
| Sand | 15·55% |
| Being fine sand | 46·86% |
| Organic and volatile matter | 7·36% |
| Clay | 30·23% |

(2) Analysis in the laboratory of the Chemical Examiner to Government—

| | |
|---|---|
| Moisture | 18·06% |
| Organic matter | 3·76% |
| Silica | 58·31% |
| Oxide of iron | 8·66% |
| Alumina | 11·21% |

Lime and magnesia, a trace.

It might at first sight appear as though the two analyses gave discordant results in regard to the proportion of clay present in the soil. This, however, is a mere matter of appearance, and is due to the fact that the method of determination was different in the two analyses. In the first the method employed was that of elutriation, which is a mechanical process of "separation of substances by washing them in large quantities of water, so that the heavier particles fall to the bottom, and the lighter ones, remaining some time suspended, are gradually deposited in a finely-divided state"[1]. What in it is styled clay is not, therefore, equivalent to chemically pure clay, but merely to a mass of very finely-divided particles to which the term mud would have been more strictly applicable. In the second analysis the method followed was the ordinary chemical one, and the proportion of clay is indicated by the amount of alumina.

Even, however, were we to accept the mud of the first analysis as equivalent to clay, which it certainly was not, the soil could not be correctly described as a clay one. In Morton's Cyclopædia of Agriculture we find the following statements regarding the classification of soils:—

Soils in general consist of a mechanical mixture of the following four ingredients:—

1. Silica, silicious sand, and gravel.
2. Clay.
3. Lime.
4. Animal and vegetable remains (humus).

There are few soils which consist of only one or two of these four substances; most contain them all, but the relative proportion of each in different soils varies considerably. A natural division of soils, accordingly, may be founded on the preponderance of one of these four chief constituents.

Upon this principle, soils may be conveniently classified as follows:—

1. Sandy soils, containing above 80 per cent. of silicious sand.
2. Calcareous soils, containing above 20 per cent. of lime.

[1] A Dictionary of Science, Literature, and Art, by Brande and Cox, 1872, p. 770.

3. Clay-soils, containing above 50 per cent. of clay.
4. Vegetable moulds (humus soils), containing more than 6 per cent. of organic matters or humus.
5. Marly soils, or soils in which the proportion of lime is more than 5, but does not exceed 20 per cent. of the whole weight of the dry soil, and that of clay is more than 20, but less than 50 per cent.
6. Loamy soils, or soils in which the proportion of clay likewise varies from 20 to 50 per cent., but which at the same time contain less than 5 per cent. of lime.

* * * * * * * *

3. *Clay-soils.*—The properties of clay-soils are diametrically opposed to those of sandy soils. Stiffness, impenetrability, great power of absorbing and retaining moisture, and great adhesiveness characterise this class of soils. They are consequently cold, stiff soils, which are expensive and difficult to cultivate. When properly cultivated, some are turned into highly fertile soils. Their faults arise out of their mechanical structure, not out of their chemical composition, which latter alone is represented in the following analyses. The former may be corrected by drainage, burning, the use of bulky manures, and the addition of lime, ashes, &c.[1]

According to this, the subsoil of Calcutta has no claim to be regarded as a clay one either in respect of its chemical constitution or its physical properties, for in the first place, even on the most liberal calculation, it does not contain anything nearly attaining the standard of clay content, and in the second it is neither retentive nor adhesive. The idea that it is retentive is probably founded on the fact that at the level at which it occurs it is in a constant state of saturation, but this saturation is not due to any special retentive property in it, but is simply the result of the very low level of the country and the great masses of water constantly entering it and serving to bring the water level even when at its lowest close to the surface. The most casual observation, however, shows that on removal from its native site the material, in place of being retentive, parts with its moisture with extreme readiness.

The following experiments illustrate the degree of impermeability which it possesses. It has been already mentioned that on drying the material becomes very hard. This hardness does not indicate any coherence under the influence of renewed access of moisture. This must be familiar to any one who has ever had experience in the excavation of deep tanks in and around Calcutta, where it is a matter patent to the most superficial observation that there is no necessity for expending labour in breaking down the hard, rough masses of the subsoil if spread out over the surface of the surrounding ground, as they rapidly melt down and disappear under the influence of the first heavy rainfall. In this respect they differ greatly from masses of peat from the beds of this material, which are frequently intercalated in the so-called blue clay. The incoherence of the dry material appears very conspicuously on immersing portions of it in water. If a fragment be dropped into a beaker full of water, an immediate and violent discharge of bubbles of air takes place and is accompanied by a rapid

[1] A Cyclopædia of Agriculture, Practical, and Scientific, edited by J. C. Morton, London, 1871, Vol. II, pp. 869—870.

process of disintegration, the mass gradually crumbling down and ultimately forming a loose sediment at the bottom of the now turbid water. The mass of air displaced during the process is evidently very considerable, and the phenomenon at once does away with any belief in the impermeability of the material. Numerous experiments were carried out with a view of more accurately determining the air content of air-dried specimens of the material. The method employed was one of displacement. The portion of soil experimented with in any case was allowed to subside gently into a glass vessel which had been previously carefully filled with water just to the point of overflow. The entrance of the mass of soil of course displaced a mass of water corresponding to its bulk. The water was caught in a glass saucer in which the recipient vessel stood, and on being measured gave the necessary datum of the bulk of the material in the dry state. As evolution of air bubbles and disintegration advanced, the water in the recipient vessel gradually subsided, and when the process had been completed, in place of standing at the overflow point, was at some lower level. Water was now added from a finely graduated measure until the overflow point was again attained and the amount necessary to do this taken as the index to the amount of air space originally present in the mass. Numerous experiments were carried out in order to test how far this method of determination was trustworthy. In these the amount of displacement caused by the introduction of impermeable bodies of ascertained bulk, such as accurately measured cubes of loaded wax, was determined, as well as the amount of addition of water necessary to regain the overflow point on the removal of the displacing body, and it was found that with a little care the amount of error was trifling, amounting only to from 1 to 1·5 c.c. when a cube of wax of 27 c.c. was employed. Allowing for errors of like amount in the case of experiments with masses of soil, the specimens were found, as a rule, to contain from 22 to 32 per cent. of air space. A material of this character can certainly have no pretension to be regarded as impermeable, and this was further proved by direct experiment.

A mass of the material was taken, and a block, measuring 14·6 c. in length, 13·0 c. in breadth, and 9·5 c. in thickness, carefully sawed out of it. This was easily done, as the mass had been kept in its original moist condition by means of keeping it constantly covered with a wet cloth. A cylindrical hollow was next excavated in one face of the block as nearly as possible 5 c. in diameter and 3·9 c. deep, which was the capacity of a glass vessel required to complete the observations; 75 c.c. of water were then introduced into the hollow and a like amount into the vessel, and the latter and the mass of soil set side by side on a table in the laboratory, the mouths of both vessel and hollow being covered by pieces of fine wire gauze. After the lapse of a period of 40 minutes the cavity in the soil was found to be empty and 50 c.c. of water were introduced. By the close of another hour this also had disappeared and 50 c.c. more were added. An hour later the cavity was almost empty and 50 c.c. were once more intro-

duced. On the following morning the cavity was found to be quite empty, the surface being still moist, but with superficial cracks on it. During the course of the forenoon 175 c.c. of water disappeared from the cavity, and the experiment was brought to a close by one-half of the moist block, which was only supported centrally by an iron tripod, breaking away, due to its own weight. During the period of experiment 350 c.c. of water had disappeared from the cavity, and only 6 c.c. from the glass vessel as the result of evaporation, leaving 344 c.c. to be credited to the permeability of the soil. This amount does not fairly represent the entire loss possible for the entire period, as the block was only under observation during the hours from 7 A.M. to 7 P.M. daily and the cavity had evidently remained empty for a long time between the experimental periods of the two successive days.

A second experiment of like nature was conducted as follows. The block of soil in this instance measured 19 c. in length, 12·7 c. in breadth, and 7·1 c. in depth, and was supported beneath on a piece of wire gauze, so as to avoid chances of breakage like that of the previous experiment. The cavity in it and the glass vessel used to determine loss by surface evaporation were of the same character as in the previous case. Daily additions of water were made to the cavity on all those days on which the laboratory was open during the hours in which it was under observation—from 7 A.M. to noon or to 3 P.M. in one or two instances. The experiment lasted for nineteen days, the water in the glass vessel having totally disappeared by evaporation on the morning of the twentieth day. The amount of water added daily to the cavity for the period was as follows :—

| Date. | Day of Experiment. | Amount of Water added. | |
|---|---|---|---|
| May 13th | 1st | 138 c.c. | |
| ,, 14th | 2nd | 135 c.c. | |
| ,, 15th | 3rd | 125 c.c. | |
| ,, 16th | 4th | 161 c.c. | |
| ,, 17th | 5th | ... | Laboratory closed. |
| ,, 18th | 6th | 116 c.c. | |
| ,, 19th | 7th | 111 c.c. | |
| ,, 20th | 8th | 124 c.c. | |
| ,, 21st | 9th | 103 c.c. | |
| ,, 22nd | 10th | 104 c.c. | |
| ,, 23rd | 11th | 103 c.c. | |
| ,, 24th | 12th | ... | Laboratory closed |
| ,, 25th | 13th | ... | ,, ,, |
| ,, 26th | 14th | 155 c.c. | |
| ,, 27th | 15th | 110 c.c. | |
| ,, 28th | 16th | 85 c.c. | |
| ,, 29th | 17th | 105 c.c. | |
| ,, 30th | 18th | 97 c.c. | |
| ,, 31st | 19th | .. | Laboratory closed. |
| June 1st | 20th | ... | Glass vessel empty. |

During this period 1,772 c.c. of water had disappeared from the cavity in the soil and 75 c.c. from the glass vessel due to evaporation, leaving a loss of 1,697 c.c. to be credited to the permeability of the soil. The entire cubic capacity of the block of soil minus the cavity was 1,635·5 c.c., and hence during the period a bulk of water had disappeared from the cavity due to percolation greater than the bulk of the entire block into which it was absorbed. The results moreover, as in the previous case, by no means represent the total absorptive power of the mass of soil for the period, as the cavity was almost always empty for a considerable period daily and on some occasions remained so for entire diurnal periods.

A third experiment was now tried with the same mass of soil and only differing from the previous one in that a glass cylinder open at both ends was fitted accurately into the interior of the cavity, any small interspaces between the glass and soil being filled with firmly rammed material, so as to secure accurate contact everywhere. The cavity in this case thus differed from those in the previous ones in having impermeable walls, so that water could only come into contact with the soil of the floor. The experiment lasted from the 1st to the 29th June with the following results:—

| Date. | Day of Experiment. | Amount of Water added. |
|---|---|---|
| June 1st | 1st | 150 c.c. |
| ,, 2nd | 2nd | 140 c.c. Cavity quite empty in the morning. |
| ,, 3rd | 3rd | 125 c.c. ,, ,, ,, ,, ,, |
| ,, 4th | 4th | 120 c.c. ,, ,, ,, ,, ,, |
| ,, 5th | 5th | 118 c.c. ,, ,, ,, ,, ,, |
| ,, 6th | 6th | 118 c.c. ,, ,, ,, ,, ,, |
| ,, 7th | 7th | ... Laboratory closed. |
| ,, 8th | 8th | 124 c.c. Cavity quite empty in the morning. |
| ,, 9th | 9th | 118 c.c. |
| ,, 10th | 10th | 119 c.c. |
| ,, 11th | 11th | 118 c.c. |
| ,, 12th | 12th | 108 c.c. |
| ,, 13th | 13th | 115 c.c. |
| ,, 14th | 14th | ... Laboratory closed. |
| ,, 15th | 15th | 107 c.c. Cavity quite empty in the morning. |
| ,, 16th | 16th | 91 c.c. Cavity contained a little water in the morning. |
| ,, 17th | 17th | 82 c.c. ,, ,, ,, ,, ,, ,, |
| ,, 18th | 18th | 75 c.c. ,, ,, ,, ,, ,, ,, |
| ,, 19th | 19th | 69 c.c. Cavity contained some water in the morning. |
| ,, 20th | 20th | 61 c.c. |
| ,, 21st | 21st | ... Laboratory closed. |
| ,, 22nd | 22nd | 90 c.c. Cavity quite empty in the morning. |
| ,, 23rd | 23rd | 64 c.c. Cavity contained some water in the morning. |
| ,, 24th | 24th | 50 c.c. ,, ,, ,, ,, ,, ,, |
| ,, 25th | 25th | 39 c.c. ,, ,, ,, ,, ,, ,, |
| ,, 26th | 26th | 43 c.c. ,, ,, ,, ,, ,, ,, |
| ,, 27th | 27th | 46 c.c. ,, ,, ,, ,, ,, ,, |
| ,, 28th | 28th | ... Laboratory closed. |
| ,, 29th | 29th | 72 c.c. |

The glass capsule registering loss by evaporation from a surface equal to that presented by the cavity had lost 60 c.c. of water during the period. The total loss from the cavity in the same time was 2,362 c.c. or 2,302 c.c. due to the permeability of the soil. The diminution in daily loss from the cavity coincident with the great increase in atmospheric humidity attendant on the onset of the rainy season comes out very distinctly. During the entire course of the previous experiment and in the early part of the present one the surfaces of the block of soil appeared continuously dry, but when the atmospheric humidity increased and the disappearance of water from the cavity conspicuously diminished, the surfaces became evidently and constantly moist. Another phenomenon, common to this and the previous experiments, but which came out most conspicuously here, was the gradual diminution in the capacity of the cavity due to the disintegrant action of the water on the soil. The floor of the cavity was originally flat and flush with the lower edge of the glass cylinder which formed its bounding wall. As time went on, however, it gradually swelled up and in place of consisting of the dense material of the mass in its original condition came to be composed of soft, loose mud derived from the disintegration of the soil at the base of the cavity and from beneath the edge of the cylinder, which ultimately, in place of resting on the firm soil, was suspended over this soft, incoherent substance.

Another experiment of a different nature was tried. In it a long glass tube was rammed with some of the soil which had been previously dried over a gas flame and thoroughly triturated. The rammed soil occupied 66c. of the length of the interior of the tube. The tube was fixed in a horizontal position with one end connected with the lower extremity of a glass receiver full of water, and the other with an open U-tube containing fragments of chloride of calcium. The experiment was initiated on the 20th May by opening the tap at the bottom of the receiver and allowing the water to pass into the tube. The water advanced along the portion of the tube occupied by the soil at a rate shown by the following figures:—

| Date. | Extent to which water had evidently advanced along the soil. |
|---|---|
| May 21st . . | 18·6c. |
| " 22nd . | 24·5c. |
| " 23rd . . | 29·1c. |
| " 26th . . | 37·5c. |
| " 27th . . | 39·6c. |
| " 28th . . | 41·2c. |
| " 29th . . | 42·9c. |
| " 30th . . | 44·5c. |
| June 1st . . | 46·9c. |
| " 2nd . . | 48·2c. |

| Date. | Extent to which water had evidently advanced along the soil. |
|---|---|
| June 3rd . . | 51·7c. |
| ,, 4th . . | 52·1c. |
| ,, 5th . . | 52·2c. |
| ,, 6th . . | 53·05c. |
| ,, 8th . . | 54·6c. |
| ,, 9th . . | 55·2c. |
| ,, 10th . . | 56·9c. |
| ,, 11th . . | 57·4c. |
| ,, 12th . . | 58·9c. |
| ,, 13th . . | 59·0c. |
| ,, 15th . . | 61·4c. |
| ,, 16th . . | 62·9c. |
| ,, 17th . . | 63·4c. |
| ,, 18th . . | 64·2c. |
| ,, 19th . . | 66·0c. |

From the above figures it appears that the water took 30 days to traverse a stratum of soil 66.c. thick, and closed, save at its extremities, by an impermeable material. Subsequent to this slow percolation continued to occur as indicated by the gradual accumulation of a considerable quantity of water within the distal portion of the tube beyond the soil.

The above experiments appear to demonstrate sufficiently clearly that the subsoil in Calcutta is just as little "impermeable" as it is "blue clay," and it now only remains to attempt to find an explanation for the erroneous ideas prevalent in regard to its nature. The peculiar colour of the material, its consistence when freshly excavated and moist, and its stony hardness on drying are perhaps enough to account for the popular description of it as consisting of "blue clay." It is, however, at first sight, difficult to understand on what the idea of its impermeable nature was founded, seeing that it must have been patent to the most casual observation that in its normal site it included a very considerable amount of water content and that it parted with this with great readiness on exposure to sunshine and dry air. The belief must apparently have been due to the fact that, whilst really containing a very large amount of air or water space, the soil is yet so fine as to prevent rapid transit of water under ordinary circumstances. Excavations made in a soil of this character will, of course, not be liable to contain conspicuous accumulations of percolated water so long as evaporation is taking place with any considerable activity from the exposed surfaces. The last of the experiments on the permeability of blocks of the soil affords a direct proof of this, for in it it was only with increased atmospheric humidity and diminished recipient power in the soil that the surfaces became conspicuously moist. In the laboratory in which the experiments were conducted there was a regular daily loss of 0·125 of an inch in depth from

exposed surfaces of water until the rise of atmospheric humidity set in, although the water was never exposed to direct sunshine and for the greater portion of the diurnal period was in a closed room absolutely protected from the influence of wind. The evaporation from surfaces freely exposed to sun and wind must, of course, be much greater under otherwise like conditions. A series of experiments on bodies of water exposed on the roof of a house in the neighbourhood of Calcutta have indicated a loss of from 26 to 37 hundredths of an inch in depth daily during the month of April. Taking this into account, along with the fine texture of the soil, we have, I believe, arrived at an explanation of the idea that the subsoil of Calcutta is impermeable. Deep excavations can only be carried out in the locality during the dry season unless pumping operations are called into play, and hence it is in the dry season only that opportunities ordinarily arise of seeing the subsoil. So long, however, as the air is dry enough and the temperature high, the daily loss by evaporation will be sufficient to do away with the amount of water constantly added by percolation, or, at all events, to do away with enough of it to prevent conspicuous accumulations of fluid, and when conditions alter, the appearance of water in the excavations is liable to be credited solely to direct rainfall and access of surface drainage.

CALCUTTA;
*The 15th April 1887.*

# On a new Genus of the Family Ustilagineæ.

BY

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(*With Plates I and II.*)

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The parasite forming the subject of the present paper was first observed in the autumn of 1886 in the Botanical Garden in Calcutta. It occurred in the leaves of *Nymphæa stellata*, *Nymphæa lotus,* and *Nymphæa rubra*, which, although regarded as a mere variety by systematists, are unequivocally, so far as physiological characters go, distinct species. It does not appear to occur either in *Euryale* or *Nelumbium.* Whilst affecting the three *Nymphæas* above mentioned, it is very much more abundant in *Nymphæa stellata* than in the others, and I shall therefore describe it in detail as occurring in that species, merely referring to any points of variation which seem to present themselves when it affects the other hosts.

In *Nymphæa stellata* it affects leaves of all ages, appearing even in quite young ones. The first indication of its presence is the appearance of small, circumscribed, bright yellow spots on the upper surfaces of the leaves (Plate I, fig. 3). These spots usually occur associated in large numbers, but occasionally perfectly isolated ones appear on otherwise healthy leaves. In some cases they are evenly sprinkled over the entire surface, but in most cases they tend to occur in patches or lines over limited areas of the leaf. Where very closely set they often become confluent at the margins over comparatively large areas. When they first appear, the alteration in the tissues of the host does not extend to the under-surface of the leaf, which retains its beautiful deep violet colour unaltered. After a time the centres of the yellow spots become brown and ultimately almost black in many instances. As this change goes on, the patches begin to appear on the lower surface as deep blue spots, which soon become greenish centrally, and eventually decay sets in and leads to the formation of perforations surrounded on the upper surface by yellow zones of tissue containing the parasite.

Before proceeding to describe the characters of the parasite it is necessary to give some account of the structure of the leaves of the host-plant. This is very peculiar in *Nymphæa stellata*, and is quite distinct from that in either of the other two species. There is an entire absence of any stratum of palisade cells,

and the lamina may be described as consisting of two layers of epidermis kept apart by enormous numbers of large idioblasts and here and there connected by bands of parenchymal tissue (Plate II, fig. 5). In the upper half of the thickness of the lamina there is a dense, sub-epidermal stratum of parenchyma interrupted by comparatively small substomatic spaces (Plate II, fig 3) and by the thick shafts of the idioblasts. In the lower half parenchymal tissue is at a minimum, only appearing in the form of thin partitions traversing a series of enormous spaces immediately above the inferior epidermis and crossed by the lower extremities of the idioblasts. The latter consist of a shaft, the upper extremity of which is in contact with the under-surface of the superior epidermis, and of a series of terminal rays, the tips of which extend to the upper surface of the inferior epidermis. In *Nymphæa lotus* and *rubra* there is a well-defined stratum of palisade cells, and the idioblasts are neither so abundant nor do they extend so completely through the thickness of the lamina as in *Nymphæa stellata.* They do not extend from one epidermal surface to the other, for while their bases are in direct relation to the under-surface of the superior epidermis, their rays, which are enormously elongated horizontally, do not reach the upper surface of the inferior one, but are spread out at some distance above it.

On making vertical sections of affected leaves of *Nymphæa stellata* the yellow patches on the surface are found to correspond with areas of tissue occupied by mycelium and spores of a parasite. The mycelium consists of very delicate, sparsely septate branched filaments, and small, simple, bud-like haustoria projecting into the interior of the cells of the host (Plate II, fig. 6). So very delicate and slender are the filaments that it is only by means of staining them that their presence in the tissue can be detected, and here, as is the case with so many fungal mycelia, Spiller's purple is by far the most efficient staining agent. The mycelium is mainly distributed in the dense, sub-epidermal parenchyma, but now and then a stray filament may be encountered in the great inferior system of intercellular spaces. Where the mycelium enters the substomatic spaces, the filaments become considerably thicker and ultimately produce masses of spores (Plate II, fig. 4), which are thus absolutely limited in distribution to the upper half of the thickness of the lamina.

The sporiferous filaments are abundantly branched and densely filled with vacuolate protoplasm. The ultimate twigs each produce a single spore, which makes its first appearance as a fusiform dilatation of the parent filament a little below the extremity (Plate II, figs. 4 and 10). The dilatation is atfirst occupied by a vacuolate protoplasm continuous with that in the distal and proximal portions of the filament. It gradually increases in size and ultimately becomes almost spherical in some cases, and as it grows it invades the terminal portion of the filament, which thus becomes reduced to a mere process connected with the distal side of the dilatation. This process is at first hollow and contains proto-

plasm, but as time goes on the latter disappears and, the cavity becoming filled up, the process is converted into a solid appendage of the dilatation. The latter becomes separated from the proximal portion of its parent filament by the formation of a septum after a great accumulation of protoplasm within it has occurred. The young spore which has thus been differentiated is at first entirely filled by a dense mass of granular matter. A gradual development of oil globules next sets in. The globules gradually fuse with one another, forming drops of progressively increasing size and diminishing number, and ultimately the process ends with the development of one great globule occupying a great part of the cavity and surrounded by a thin layer of finely granular protoplasm. Whilst these changes have been progressing in the contents of the spores, a very considerable thickening of the walls has also occurred and the mature spores appear as nearly spherical cells with thick walls, a projecting beak, and oily and protoplasmic contents.

The ripe spores are colourless or have a faint brownish tinge due to a slight darkening of the thick epispore. The beak is always colourless. Where they become detached they not unfrequently carry a portion of the empty parent filament with them. They are of considerable size, measuring about $14\,\mu$, exclusive of the beak, in length by 10 or $11\,\mu$ in breadth. The beak is about $1{\cdot}8\,\mu$ in length. As they accumulate in the substomatic spaces they frequently so fully occupy the cavities as to form dense masses in which the individual spores are closely pressed together and to the boundaries of the spaces (Plate II, figs. 1, 2). When stained with eosine alone the epispore takes a bright orange-red tint, whilst the contents become strong rose. Spiller's purple dyes the filaments and spores deep violet, apparently due to staining of the cell walls. The spores germinate *in situ*, and the long promycelia emerge on the surface of the leaf, not due to any rupture of the epidermis, but by passing out through the stomata (Plate II, fig. 15). Due to this the central darkened portions of the patches occupied by the parasite become covered by a series of tufts of colourless filaments which are readily recognisable under examination with a simple lens.

The phenomena attending germination of the spores *in situ* or in water are as follows :—A gradual solution and disappearance of the great oil globule takes place and the contents once more become evenly granular. A thick germinal tube now emerges at some point in the lateral surfaces of the spore (Plate II, fig. 8). This rapidly elongates, and, as it does so, the protoplasmic contents of the spore gradually travel into it and continue to advance along its course, leaving the lower part empty and traversed by septa. The germinal tube thus eventually comes to consist of three or four superimposed cells. The distal extremity next comes to show a number of short, thick branches, with rounded extremities (Plate II, fig. 9). The protoplasm continues to advance into these and ultimately nearly leaves the germinal tube, only a small

quantity remaining close to the upper extremity of it. The number of processes which thus come to form a capitulum at the end of the tube varies from four to six as a rule. They gradually elongate and assume a slender fusiform outline with a pointed extremity, and, as they do so, they become separated from the germinal tube by a septum and traversed in their length by two or three others (Plate II, fig. 7). Each process thus comes to consist of three or four cells. The terminal pointed one next gives origin to two or three minute sterigmata at its apex, and each of these in its turn develops a long slender sporidium (Plate II, fig. 16). These conjugate just as the sporidia of *Tilletia* or *Entyloma*, union as a rule taking place between sporidia of distinct processes (Plate II, figs. 11, 12, 13). Conjugation takes place both before and after detachment of the sporidia from the promycelium. The uniting tube usually is situated towards one or other extremity of the sporidia and is often of considerable length and curiously sinuous course (Plate II, fig. 14). The sporidia are full of finely molecular protoplasm and are extremely long and slender, measuring in many cases as much as 21 *u* in length by 0·9 *u* in breadth. After conjugation has occurred the greater part of the protoplasm appears to pass into one or other of the united bodies which grows considerably longer, and at the same time transverse septa appear in it and in the other one. Finally, secondary sporidia are given off laterally from the primary ones, varying in form in different instances, in some cases being very short, in others elongated and very similar to their parents.

When the spores are cultivated in a nutritive fluid, such as strong decoction of *Nymphœa* leaves, the phenomena attending germination differ in certain points of detail. All the earlier phenomena are of the same character as those which have just been described up to the development of the primary sporidia. After this deviations from the previous type tend to occur. Conjugation is by no means of such constant occurrence, and formation of secondary sporidia frequently occurs from unconjugated primary ones (Plate II, fig. 12). The secondary sporidia, which here appear to be always similar in form to their parents, in their turn give origin to a new series of slender spindles, and, the same process being continued, ramified series of filaments, built up of connected spindles, gradually arise. The phenomena are, in fact, precisely parallel to those described by Brefeld as attending the cultivation of the spores of other forms of *Ustilagineœ* in nutritive fluids in place of water.[1] The capacity for continued saprophytic in place of parasitic existence affords the only explanation of the persistence of the parasite from year to year in many cases; for example, where it is present in tanks containing *Nymphœa lotus* and *Nymphœa stellata* alone, there are periods of many months during the cold and hot seasons in which not a single leaf, or trace, of the host-plant is present to afford accommo-

[1] Botanische Untersuchungen über Hefenpilze Von Dr. Oscar Brefeld: Leipzig, 1883.

dation to the parasite. In bodies of water containing *Nymphæa rubra* there is certainly never such an entire disappearance of the host-plants, but there is at the same time a seasonal disappearance of the parasite. Where however, as in the case of the other two *Nymphæas*, there is prolonged continuous absence of the host-plants, the parasite would necessarily die out if only capable of parasitic existence, for the spores have not the character of resting cells, but germinate at once on attaining maturity. The continuous development of conidia by yeast-like multiplication originating with the sporidia tides over the period of absence of host-plants and provides for the infection of the latter when they again make their appearance.

The parasite when occurring in *Nymphæa lotus* and *Nymphæa rubra* presents the same general features as in *Nymphæa stellata*. The spores are not, however, so exclusively limited in their distribution to the substomatic spaces, but not unfrequently occur also in the large cavities immediately above the inferior epidermis. In *Nymphæa lotus* the areas affected by the fungus appear as yellow spots and patches like those in *Nymphæa stellata*, whilst in *Nymphæa rubra* they appear as pinkish spots in the dark-red surface of the leaves. Where such spots are very abundant the presence of the parasite often appears to exert a very special action on the tissues of the host, the chlorophyll in the cells of the affected areas persisting after decay has set in around. This phenomenon is not peculiar to this parasite, but often appears very much more conspicuously in others; for example, when the leaves of *Albizzia Lebbek* affected by *Ravenelia* undergo the ordinary seasonal change of colour preparatory to falling off, the patches of *Ravenelia* appear as conspicuous brilliant green spots on the general bright yellow surface and remain as such long after the actual fall of the leaf due to persistence in the chlorophyll content of the host-cells of the affected areas. The phenomenon almost would appear to indicate the presence of a lichenoid symbiotic relation between the fungal and host tissues in place of a merely parasitic one.

When we come to consider the nature of the parasite, we can have no hesitation in referring it to the *Ustilagineæ*, but the question of its precise place in the family and of its claim to be regarded as belonging to a distinct and new genus still remains to be discussed. The peculiar characters of the promycelium and the terminal development of the sporidia at once indicate a close relationship to both *Tilletia* and *Entyloma*, and, as the ripe spore mass is neither pulverulent nor dusty, specially to the latter genus. There are, however, certain distinctive features present which are, I believe, of generic value. These are, first, the subterminal development of the spores; and, second, the development of the sporidia, not directly on the tip of the germinal tube, but on a series of intermediate structures which occupy the place of the sporidia in *Tilletia* and *Entyloma*. The fact that conjugation never occurs in the bodies at the tip of the germinal tube is sufficient to show that they are not to be regarded as

corresponding to sporidia, but as portions of the promycelium, and the constant occurrence of conjugation in the bodies developed from them shows that these really are primary sporidia, and not secondary ones derived from such bodies. The promycelium is thus much more complicated than in either *Tilletia* or *Entyloma*, consisting not merely of a simple septate germinal tube, but of such a tube and a series of septate terminal branches. The peculiar rostrate character of the spores is also very distinctive, but it is of course a mere result of the fact that the spores are here developed subterminally, and not intercalarly, as they are in *Entyloma*. The above characters appear to entitle the parasite to be regarded as forming the type of a new genus which I propose to name and define as follows :—

*Rhamphospora* (Family USTILAGINEÆ).

Spores isolated ; produced subterminally ; beaked ; promycelium consisting of a long germinal tube with terminal branches bearing apical sporidia.

*Rhamphospora Nymphœa.*

Characters those of the genus ; habitat, the leaves of *Nymphœa stellata, Nymphœa lotus,* and *Nymphœa rubra.*

CALCUTTA ;

*The 15th April 1887.*

# On an Entophytic Alga occurring in the leaves of Limnanthemum indicum, with notes on a peculiarly parasitic variety of Mycoidea.

BY

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(*With Plates I and III.*)

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The Entophytic alga described in the following pages was first observed in the leaves of *Limnanthemum indicum* in tanks in the Botanical Garden, Calcutta, in October 1886. At that time it was extremely abundant in some tanks and caused very conspicuous effects in the leaves of its host. The infected leaves showed patches of yellowish discolouration of the upper surface, sometimes isolated, sometimes in groups and slightly elevated above the level of the surrounding normal tissue (Plate III, fig. 1). On removing the leaves from the water the discolouration was found to be confined to the upper surfaces of the leaves, and on examining the affected areas under simple lens, they were found to differ from the surrounding ones in containing great numbers of large, brilliant green rounded bodies situated beneath the epidermis. On making thin horizontal sections from the surface of the leaf and examining them under somewhat higher powers, these bodies were found to be large, oval or spherical cells situated in the substomatic spaces. In the discoloured patches such cells were present in immense numbers and so closely distributed that almost every substomatic space contained one and in some cases two of them (Plate III, fig. 2). They were not, however, absolutely confined to the conspicuously discoloured areas, as isolated specimens frequently occurred at wide intervals over the apparently normal surfaces around. The individual cells varied greatly in the character of their contents, some having a great, central, clear, colourless area surrounded by a clear pale green peripheral zone containing a few starch-like corpuscles, others being deep green and granular throughout, others also deep green, but with the contents presenting a mulberry-like nodulated appearance and still others in small numbers of various tints ranging from pale yellow to strong reddish orange and densely filled with large amyloid bodies. At first sight they presented so many features resembling those of Cohn's Chlorochytrium Lemnæ that it appeared probable that they belonged to the same

genus, but on a study of their life history it was evident that this was not the case, but that they were representatives of a closely allied and apparently as yet undescribed one.

The leaves of *Limnanthemum indicum* in which they occur are characterised by the enormous number of stomata in the superior epidermis and by the presence of comparatively large substomatic spaces between the subjacent palisade cells (Plate III, fig. 4). Due to this the tissue immediately beneath the superior epidermis is of an extremely open, almost reticulate character (Plate III, fig. 6). The algal cells are absolutely confined to the substomatic spaces which they deform in various ways as they increase in size and press upon the surrounding tissue elements. Beyond the distortion arising in this way, the presence of the foreign bodies produces no appreciable effect on the tissues save causing a permanent wide patency of the stomatic orifices. There is no sign of injury of the surrounding palisade and ordinary parenchymal cells, which in general aspect and amount of chlorophyll content differ in no respect from those in areas which have not been invaded by the alga. Due to the mutual pressure of the algal and host cells many of the former in many cases gradually lose their originally evenly rounded contour and come to present irregularly lobed or even angular outlines. They naturally vary very considerably in size according to the stage of development, but mature green specimens give an average of 59 $\mu$ by 50 $\mu$. On making vertical sections of the affected leaves the relations of the algal cells to the surrounding elements appear very clearly. They press laterally on the neighbouring palisade cells and above are in close contact with the stomatic openings and the under-surfaces of their guard cells (Plate III, fig. 5).

The cells differ greatly in appearance in different cases, as has been already mentioned, and this difference is connected with their age and with the ultimate developmental changes which they undergo in connection with seasonal influences. Young cells have a delicate cell-wall surrounding a thin layer of pale green protoplasm within which is a great colourless area containing a single nucleus. Amyloid granules are present in gradually increasing numbers in the peripheral green layer (Plate III, fig. 3). The green colouring increases in intensity and extends more and more into the central portion, and the contents thus gradually become of a uniform deep green and of coarsely granular consistence. On removing the colouring matter with alcohol and subsequently staining the presence of a single nucleus is still demonstrable up to this stage. Repeated processes of nuclear division now set in and the contents are thus gradually converted into a continuous mass of protoplasm, including a very large number of distinct nuclei (Plate III, fig. 8). A differentiation of the cytoplasm now occurs and each nucleus becomes gradually invested by a separate layer proper to itself, the entire mass of contents as the process goes on coming to present a lobulated, mulberry-like appearance. The nucleated protoplasts which have

thus arisen are zoospores. They soon begin to show movements and the cell becomes occupied with a dense mass of swarming bodies. Rupture of the cell-wall now occurs and the zoospores emerge in rapid succession and swim off actively in the surrounding fluid. The process of emergence does not seem to be limited to any special period, or, at all events, the period is an extended one, as emergence has been observed to occur from early in the forenoon to late in the afternoon.

The zoospores are biflagellate, and on emergence are pear-shaped with the broader portion of the body bright green and the narrow anterior flagellate area colourless (Plate III, fig. 9). It is of course difficult to obtain accurate measurements of such active bodies, but, so far as could be ascertained, they measure about 7 to 8 $\mu$ in length and about 5 $\mu$ in breadth. They swim about very actively with a combined movement of rotation and progression. The body contains several large, refractive particles, probably of amyloid nature. Large numbers of them conjugate to form zygozoospores (Plate III, fig. 11). In this process two of the zoospores approach one another closely, their anterior pointed extremities come into contact and a gradual process of fusion spreads backwards along the bodies until complete union has been effected. In exceptional cases three, in place of two, zoospores unite in this fashion. The normal zygozoospores are, of course, considerably larger than the zoospores, and are generally more or less spherical, even from the outset. They are provided with four flagella and continue to move actively about for some time. The movements, however, soon decrease in energy, the body ceases to progress freely through the water; it settles down and after keeping up a tremulous movement for some time becomes quite motionless. Parallel phenomena of change of form and cessation of movement occur in those zoospores which do not conjugate. All the zoospores developed in a zoosporangium do not always effect an exit, but a certain number remain behind, and after swarming actively for some time gradually become spherical and motionless (Plate III, fig. 10). Such motionless, retained zoospores measure about 7 $\mu$ in diameter. After settling down apart from leaves of *Limnanthemum* or on the general surface of the leaves the zoospores show no further developmental change. They never germinate like those of *Chlorochytrium* and soon lose their green colour and disintegrate. The actual process of invasion of new portions of leaves has never been observed, but from the appearances present in various cases there can be no doubt that the zygospores and probably zoospores also gain access to their sites of further development by direct transit through the stomatic orifices. In many cases the latter can be seen to be as it were plugged by bodies which have failed to secure entrance, while in others circular bodies, identical in characters with the still zygozoospores and zoospores, can be detected in the substomatic spaces. From the dense aggregation of large groups of zoosporangia which so frequently occurs it would appear that, as a rule, the zoospores do not travel far, as it

is impossible to explain the grouping except as the result of invasion of an area close to the site of discharge of the zoospores.

The phenomena which have just been described are characteristic of those periods of the year in which the alga is in full activity, during the rains and beginning of the cold weather. As the cold weather advances, however, more and more cells tend to assume a resting condition, and ultimately all persisting ones doing so, the alga disappears for the season from the leaves of the host-plant, the old leaves containing the resting cells gradually dying off and there being no source of infection for those which replace them. The passage of the cells from the active to the resting stage is attended by the following phenomena. A very great accumulation of starch takes place, and at the same time the colour of the cell-contents gradually changes from deep green to yellow, and ultimately to deep reddish orange. The character of the cell-wall also changes. It becomes greatly thickened and often very irregularly, so that it no longer presents an even outline, but is provided with a series of irregular projecting masses (Plate III, fig. 7). The contents appear to undergo a process of condensation and shrink away from the cell-wall, and as they do so they become invested by a separate bounding stratum of considerable thickness. In old resting cells there is frequently a more or less pronounced brown colour due to browning of the thick outer cell-wall. They vary very considerably in size, but have an average measurement of 34 by 38 $\mu$. The details of measurement obtained from an isolated cell in the sediment deposited in a vessel containing infected leaves were as follows: length 55 $\mu$, breadth 36 $\mu$, thickness of the coats varying in different parts from 4·7 to 9 $\mu$, diameters of the orange mass of contents 43 by 31 $\mu$. In certain cases the development of resting cells is imperfect, and after a great accumulation of amyloid corpuscles has occurred there is no development of yellow or orange colouring matter, but a mere process of fading and disappearance of the chlorophyll, so that the cell is ultimately reduced to a sac containing a colourless mass of starch grains. After the resting cells have once been fully developed they remain unaltered for months, and as the leaves containing them die off and decay, they become free in the sediment of decomposing host tissues. During the cold-weather months they remain unaltered, and on the onset of the hot season only do they begin to change. The colour gradually changes from orange to green, the starch grains for the most part undergoing solution at the same time, and cells come thus, save in respect of the thickness of their walls, to resemble ordinary zoosporangia. A formation of zoospores next takes place, and on the emergence of these a fresh succession of active generations recurs, until the approach of the cold weather heralds in a gradually increasing production of resting cells.

The alga, whose life history has just been described in certain points, very closely resembles Chlorochytrium, as originally described by Cohn[1] and sub-

[1] Beiträge zur Biologie der Pflanzen. Zweites Heft. 1872, S. 87.

sequently by George Klebs.[1] At the same time, however, it presents certain very distinctive features separating it from that genus. The most important of these distinctive points are, first, the fact that the zygospores and zoospores do not germinate as the zygospores of Chlorochytrium Lemnæ and the zoospores of Chlorochytrium knyanum do; and second, that the zoospores become free within the zoosporangium in place of being discharged from it in a common gelatinous investment from which they are subsequently freed, as is the case with those of Chlorochytrium. Taking these facts into consideration, I would propose to refer the present alga to a distinct genus, Stomatochytrium. The definition of the genus and species may stand as follows :—

*Stomatochytrium* (Family PROTOCOCCEACEÆ).

Entophytic : zoospores conjugating ; neither zoospores nor zygozoospores germinating; zoospores liberated within the Zoosporangium.

Stomatochytrium Limnanthemi: characters those of the genus; habitat, the substomatic spaces in the leaves of *Limnanthemum indicum.*

The characters and life history of this organism appear to me to be very instructive in connection with the true parasitism which was ascribed to Chlorochytrium by Cohn and to various other similar algæ by other observers. The phenomena presented by Stomatochytrium appear very strongly to support the views of Klebs, who maintains that in such cases we have not to do with true parasitism, but merely with entophytism. In the present instance there is absolutely nothing to show that the intrusive elements abstract organised materials from the host tissues—nay more, there is absolutely nothing to show that they abstract materials of any kind from them. As has been already pointed out, the presence of the algal elements fails to produce any appreciable effect on the surrounding tissue elements beyond displacements and distortions arising as the result of mechanical causes. In this case, too, there is no injury to the integrity of the tissues connected with the access of the algal elements to their site of subsequent development, as there is in the case of Chlorochytrium, for the substomatic spaces are directly invaded by means of the stomatic orifices. Everything here points unequivocally to the correctness of the view maintained by Klebs, that in such cases the algæ only differ from common ones in that they have gradually become specially adapted to living in specially-protected sites, the protection afforded by the surrounding masses of host tissue and not any derivation of nutritive materials from them being the real determinant of the entophytic habit. There is certainly in Stomatochytrium no evidence of any abstraction of nutritive materials from the host tissues. At the utmost we are dealing with a diversion of nutritive materials which ought properly to have been devoted to the host, and not with any abstraction of materials which have ever formed a real part of the host, and when the very

[1] Botanische Zeitung, 1881, Nos. 16 to 21.

superficial distribution of the intrusive elements and the permanent and wide patency of the stomatic orifices is taken into account, it appears to be very doubtful whether any diversion of nutritive materials takes place to any appreciable extent, and whether the nutritive processes are not carried out by the algal cells precisely as they are in the case of algæ attached to the common external surfaces of leaves in place of, as in the present instance, being located in what may be regarded as mere involutions of these surfaces.

When dealing with this question of parasitism and entophytism of algæ, Klebs points out that Mycoidea parasitism affords the most striking example of apparently true parasitism in algæ which is yet known, and during the course of the past season I have met with a variety of the plant in which the parasitism appears to be unequivocal. It occurs on the leaves of *Cinnamomum iners* (Reinwardt), and is very abundant on the specimens of that tree in the Botanical Garden, Calcutta. The affected leaves show conspicuous circular black patches surrounded by narrow yellow areolæ and penetrating the entire thickness of the lamina (Plate I, figs. 5, 6). Such patches are present in small numbers on a large number of leaves, and now and then cover the greater part of the surfaces. There is little or no elevation of the surface of the discoloured patches above the general surface of the leaf, as there is in the case of common subcuticular patches of Mycoidea. The size of the patches naturally varies greatly with age, ranging from 0·03 inch to 0·69 inch. As they increase in age the centres of the patches become greyish, dry, and friable, and ultimately disintegrate, giving origin to perforations penetrating the entire thickness of the lamina and surrounded by black and yellow rings. If the black areas of the patches be carefully examined during the hot weather or rains, they may frequently be seen to present prominent specks which on the upper surface of the patch appear reddish and on the lower one pale yellow, and by the aid of a lens these are resolved into tufts of fertile filaments of Mycoidea with the normal terminal zoosporangia. The filaments and zoosporangia emerging on the lower surface of the patches do not merely differ in colour from those emerging above, but are also considerably smaller. The difference in size and colour of these inferior elements is indeed so striking that were they alone examined, it would appear as though one were dealing with a new species of Mycoidea, but on examining the filaments and zoosporangia on the upper surface it becomes at once evident that this is not so. The filaments emerge from the surfaces of the patches in little isolated tufts, and there is no general rupture of the epidermal tissue. In small, young patches the tufts are situated centrally, but as time goes on and the patches continue to spread peripherally, they no longer appear in the centre, but are arranged at intervals along a circular zone which gradually passes more and more outwards. It is a curious fact that in these leaves which are so abundantly affected by this peculiar form of Mycoidea, the common comparatively superficial variety of the alga seems very rarely to occur save to

a very limited extent in initiating the deeper affection. The leaves in some cases show an abundance of superficial primary discs like those from which the common secondary discs originate. A certain number of secondary subcuticular discs may also occasionally be detected, but these are ordinarily of very small size, as the majority soon penetrate deeper to give origin to the characteristic patches. When they do so they remain for a time recognisable in relation to the patches, but soon dry up and entirely disappear.

In determining the distribution of the algal elements throughout the affected areas, the principal difficulty lies in the fact that the dead host tissues are so darkly coloured that, unless sections are very thin, they are opaque, and so brittle that thin sections are extremely liable to break up into fragments. The results obtained from the examination of a very large number of sections were as follows:—Beneath the epidermis over the general surface of the upper side or the affected area, there is a fairly evenly distributed layer of algal cells which separates the epidermis from the subjacent stratum of palisade cells (Plate I, fig. 8). There is as a rule no such general thalloid algal stratum beneath the inferior epidermis, but merely scattered masses of algal cells penetrating to a considerable depth into the substance of the lamina and giving off emergent filaments, which rupture the epidermis and appear on the surface (Plate I, fig. 9). The thalloid stratum beneath the superior epidermis is very thin over the greater part of its extent, consisting of one or two strata of cells only, but here and there it increases in thickness, and at these points tufts of emergent fertile filaments arise. In some cases the algal cells are of green colour, but in many cases, especially in the inferior masses, there is not a trace of green present and the contents are of a brilliant yellow. The distribution of the superficial masses of algal elements, the conspicuous changes in the tissue of the lamina throughout its entire thickness, and the presence of emergent fertile filaments on both surfaces, clearly show that the alga must penetrate the leaf throughout, but it is very difficult to obtain direct evidence of the fact, and it was only after preparing a very large number of sections that specimens were obtained satisfactorily demonstrating it.

The tissue of the leaves is extremely dense and the thick walls of the cells acquire a very deep brown colour in the central portions of the affected areas, the contents at the same time shrinking and becoming reduced to brown amorphous masses and particles. In the peripheral parts, corresponding with the yellow superficial areolæ various stages of advance towards the conditions present in the centre are visible. The cell-walls are yellowish and their contents more or less modified, the chlorophyll gradually disappearing, and the protoplasm becoming lumpy and granular. In many places, even in the thoroughly altered central areas, peculiar circular, brilliant green bodies are present (Plate I, fig. 4) in considerable numbers among the dead and discoloured tissue elements, but whether these are really algal cells or mere products of the alteration in the host

tissues it is hard to determine. At certain points in some specimens, however, continuous penetration of the lamina throughout the greater extent of its thickness by unequivocal algal elements connected with those of the superior subepidermal thallus can be recognised, the peculiar green or yellow colour of the contents distinguishing the parasitic from the host elements very clearly (Plate I, fig. 7). It appears that, as a rule, penetration of the deeper portions of the laminar tissue only occurs at isolated points by means of series of cells which serve to connect the inferior surface of the superior, subepidermal thallus with the scattered masses of cells above the inferior epidermis giving origin to the inferior tufts of fertile filaments.

We have here to deal with intrusive algal elements which, both as regards their relation to the host tissues and the effects which they produce on them, clearly differ greatly from those of Stomatochytrium. The algal elements here do not occupy normal interspaces in the tissue, but force their way between normally closely adapted structures breaking up the continuity of the laminar structure. They produce effects on the tissues of the host not limited to mere distortion from mechanical pressure, but giving rise to such interference with the normal process of nutrition as to lead to death, and ultimate disintegration in masses of tissue in which mere mechanical distortion is entirely absent. The effects are certainly characteristic of parasitism as distinguished from simple entophytism. It is impossible to suppose that in this case the nutrition of the intrusive elements can be carried on without interfering with that of those of the host. They are not, like those of Stomatochytrium, so superficially situated as to have a direct relation to the outer world. On the contrary, they are, save where fertile filaments emerge, buried in dense masses of tissue. Due to their distribution they must at all events interfere with the nutrition of the host elements by misappropriation of materials properly destined for the use of the latter. Whether the interference goes further than this, whether it advances to the stage of appropriation of organised products developed by the host tissues, remains uncertain, but the instances in which large masses of the intrusive elements appear to be almost or totally devoid of chlorophyll and yet in vigorous growth strongly suggest that such higher parasitism really prevail.

CALCUTTA;
*The 15th April 1887.*

# The Fly-catching habit of "Wrightia coccinea."

BY

SURGEON A. TOMES, M.D.,

BENGAL MEDICAL SERVICE.

(*With Plate VI.*)

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In a neighbour's garden at Midnapore are two fine shrubs of *Wrightia coccinea.* They were in full bloom in April and May, and their handsome deep red waxy-looking flowers were often observed to hold, entrapped, one or more living and struggling flies.

Learning from Dr. King (who kindly identified the species for me) that the peculiarity in question does not appear to have been noted by botanists hitherto, I have been induced to investigate the mechanism of the trap with a view of discovering the meaning of the habit.

The flowers of this *Wrightia* have a very strong attraction for insects. This is owing to their gaudy colour, to their possessing a peculiar heavy vinous smell, and to the secretion of a syrupy liquid which bathes their internal surfaces. So strong is the attraction that while I was engaged in the dissection of a specimen, a portion of the perianth lying on my table was promptly discovered, seized, and carried off by a hungry cockroach.

The insects found entrapped were almost invariably common house flies caught by the proboscis, but occasionally a rather large species of ant caught by the neck, in a trap formed by the stamens (see Fig. 1, pl. VI).

On certain days dozens of flies would be caught thus, while on others not one would be seen.

On one occasion a single flower had succeeded in imprisoning four flies all at once, while two flies were often to be found in the same flower.

The insects after capture were not digested, but died a lingering death, and were devoured by red ants, which appeared to swarm over the tree. A fly which I released from the trap after a long detention seemed to be none the worse except for a bruised and swollen proboscis.

The "trap" is formed by the andrœcium in this way:—The flowers are pentandrous; the 5 stamens, somewhat arrow-headed in shape, are of strong woody consistence, and each is bordered by a stiff narrow wing or membrane (see Fig. 3, pl. VI, a and b). They spring from the perianth, and converging surround the pistil in the form of a cone, closely embracing it at its upper part.

For the upper half or two-thirds of their length they are closely in contact with each other, laterally, by means of their winged edges (see Fig. 3, pl. VI), but they are separated below by narrow slit-like openings, while at the base of the cone below the tips of the wings and between the stout filaments are left 5 quadrangular openings of considerable size, through which the nectary can easily be reached.

In every cone there are thus 5 slits, each of which constitutes a separate trap for insects.

If a thread, a fine straw, or a bristle, imitating the proboscis of a fly, be introduced into the interior of the cone through one of the basal openings and then gently withdrawn in an upward direction, it will readily pass into the slit, where, as traction continues, it soon becomes fixed. If now the hand be raised still higher, it will be found possible to extract the bristle very slantingly through the apex of the cone, where the points of the anthers give a little, and in the process of withdrawal it comes into contact, first, with the sticky stigmatic tissue, then with the pollen, some of the grains of which will be found adhering to its point.

It seems to me that the action of the trap is purely mechanical. After repeated experiments I could not discover any appearance of irritability or sensitiveness of the stamens. Irritation of the interior of the cone had no effect in the closer approximation of the adjacent wings, the width of the slit remained the same even after capture of an insect. The slit-like interval between the wings narrows from below upwards until the sides actually touch. The wings themselves are "set" somewhat inwards towards the long axis of the cone (Fig. 6, pl. VI). They thus tend to fall together valve-like and the whole arrangement is adapted to favour the wedging in of an impacted proboscis. There is plenty of room for an insect to withdraw its head or proboscis through the large opening at the base, and it is not easy to understand why entanglement should so often occur.

Possibly while in the act of *searching*, the member becomes *jammed*, and once caught in the slit all efforts except in one direction, directly upwards, as has been shewn, only cause it to be held the tighter; so tight, indeed, does the trap hold that once the struggles of a captured ant were seen to result in its decapitation.

The free edges of the wings are smooth and hard. A lens reveals no teeth or ridges, but they are seen to be finely ribbed longitudinally.

The shrubs have now ceased to blossom and want of material prevents further enquiry at present. From the foregoing the plant clearly is not insectivorous, and there is a strong probability that the fly-catching habit is accidental, resulting from the arrangements to secure the fertilization of the species. In the two specimens to which I have had access all the flowers were hermaphro-

dite, with perfect male and female organs, but their construction is such that self-fertilization cannot easily take place. The anthers are introrse and dehiscence apical, but the upper portion of the cone is so closely applied to and around the stigma, that pollen could scarcely fall spontaneously upon the stigma; in fact self-fertilization could only be effected at a mechanical disadvantage. On the other hand, all the circumstances of the case appear to favour the idea of fertilization by insect agency. But flies and ants are obviously not destined to be the agents; these are caught and perish, while stronger ones, such as bees for instance, or butterflies and moths, perhaps having a longer proboscis, may be able to release themselves (and a long proboscis would be an advantage for withdrawal through the apex of the cone) and so to carry away the pollen to the next flower they visit. The fact that the stigma must be reached before the pollen in the process of withdrawal supports this view. Pollen adhering to the tip of a proboscis after its extraction from one trap would certainly be wiped off upon the stigma of the next one in which it may chance to be arrested. Such are the points that I have observed in connexion with this apparently wanton destruction of insect life by the *Wrightia*, and the interpretation they appear to suggest, but I by no means claim to have finally solved the mystery, for I admit that I have never actually seen a bee or other larger insect engaged in the flower, or in the act of releasing itself from the trap.

# Note on the Occurrence of a Minute Blood-Spirillum in an Indian Rat.

BY

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BRIGADE SURGEON, BOMBAY.

(*With Plate IV.*)

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The following memoranda relate to two separate subjects, either of which may be sufficiently interesting to warrant attention. One of these fresh data is the detection of a blood-parasite hitherto, I think, unrecognised; and the other is the demonstration of the possibility of introducing into the circulation of a monkey the hæmatozoon so often found amongst Indian rats—*vide infra* experimental series F., No. 2. In recording this last-named fact I have taken the opportunity of mentioning other experiments and observations independently acquired, and also of commenting on materials already acquired regarding the pathology of the remarkable horse-disease known in India as 'surra' or 'the relapsing fever of equines.' Some Charts and Illustrations are appended.

NOTE NO. 1.—Whilst examining in August 1885 the fresh-drawn blood of a rat (*Mus decumanus*) with a $\frac{1}{10}$ inch water-immersion lens, I noticed a quick-twirling movement amongst some of the red discs, which reminded me of the motion produced by the human febrile blood-spirillum (*Spirochæte Obermeieri*, Cohn) and which was eventually found to be due to the presence of a similar yet much minuter organism. In its living state this body is not easily examined, on account of its small dimensions, its vivacity, colourlessness and translucency; but in dried and aniline-stained preparations it becomes distinct as an extended and uniformly slender filament of clearly spiral construction, having a length commonly somewhat less than the diameter of a red blood-disc, but varying from 5· to 9· $\mu$, equivalent to about the $\frac{1}{5000}$ to $\frac{1}{3000}$ of an inch, and according to its length presenting from 4 to 8 close spiral turns. The organism is exceedingly slender, and both of its ends seem a little pointed; at neither end, however, could I, with an English P. and L. $\frac{1}{12}$ inch oil-immersion detect a flagellum. The structure appeared homogeneous throughout,—*vide* (Plate IV, figs. 1 and 2). If, before staining, the dried specimen of blood be rendered translucent by strong acetic acid or solution of potass, a clearer view of the organism

may be obtained, without any more manifest alteration of its aspect than a slight thinning.

The number of spirilla present was always moderate—2, 3, or 4 at most being usually visible in the field at one time; but after dissolution of the obscuring red discs a somewhat larger number may be brought into view, and only by such method can an accurate estimate of the total be made. If only 50 organisms occur in a drop of the rat's blood, the entire number in the circulation would amount to several thousands. Though sometimes I failed to detect at once any organisms, yet, as a later search might prove successful, or a scrutiny by the clearing method just alluded to, I was led to infer that possibly some few commonly existed in the blood. As occurs with the human spirillum, this form showed a tendency to resort to the open serum spaces left on coagulation; but on the other hand there was not then seen a disposition to join or cluster in close groups, nor did the red discs seem to be especially attractive to them. Their movements in the fresh-drawn blood were very active and tolerably sustained, consisting of rotation round the long axis, propulsion either forward or backward, and occasionally an energetic lateral twisting or lashing. It seemed as if during movement a slight unwinding of the screw, with consequent elongation, also took place. When stationary, the filaments are extended and the spirals distinct; simple rotation may occur without any other change in position. Owing to the varying obliquity of direction assumed, much variety of aspect ensues; and a mere dancing dot—the tip of a vertically submerged filament—may for a time be the only visible sign of its presence.

In hermetically sealed specimens of blood, either pure or diluted with half-per-cent. salt solution, the spirillum may be watched at leisure for a period of over 24 hours. Its movements become languid and more rotatory, the form rigid, and the spirals closer set: with quiescence at the end of 12-24 hours or more, the filaments may assume a dotted aspect, which did not change so long as detectable, but at this time observation becomes difficult, and after repeated efforts I was unable to trace unequivocal signs of spore-production: in the course of 40-50 hours the organisms ceased to be visible. The temperature of the air ranged from 78° to 85° F.

The blood for examination being obtained by puncture of the skin (at toe or root of tail), the admixture of adventitious particles was inevitable, and, in consequence, bacteria soon made their appearance in the sealed specimens watched.

That the filaments under consideration are defined living organisms was sufficiently self-evident; and under the circumstances of enquiry, they might have been regarded as possibly either incipient monads of the usual kind, or as a form of streptococci. Yet, on the one hand, I never perceived any trace

of a transition towards the flagellate hæmatozoa of the rat; and on the other, undoubted micrococci did not appear in any of my specimens until after the lapse of 6-12 hours, and these were of diverse aspect and far less active amidst the red discs. Alongside such later oscillating coccus-chains this spirillum might be seen stretched and still, and obviously quite dissociated. Bacillar rods motile or quiescent were distinguishable readily enough, and the displacement of the blood-discs which they caused had not the peculiar thrilling character here noted. (*See* Plate IV, fig. 2.)

From its special aspect, its movements and behaviour with re-agents, I infer that the organism in question is a *bacterium* belonging to the genus Spirochæte—or in other words a flexible and unfolding spirillum, such as from its small dimensions might be named provisionally *Spirillum minor*, with an habitat in the blood of an Indian rat, where it is rare, *e.g.*, once found in 117 individuals. It is five to ten times shorter than the *S. Obermeieri*, and also slenderer. Respecting its biological features, these also are strikingly divergent, indicating a difference of properties and relationship to the common blood-medium. Thus, first, under similar experimental conditions this minute hæmatophyte does not, contrary to the pathogenetic bacterium, display any tendency to grow in the blood after withdrawal from the body: it does not then, however, comport itself like the ordinary putrefactive microphytes, but, as already stated, after quiescence so long as watched seems gradually to decay. Next, as to pathogenetic properties, an association with pyrexia of a relapsing type was not demonstrated in this case: for although the rodent-host when first seen was decidedly feverish, yet a possible cause for the pyrexia was some attendant traumatism, and when the fever subsided it was not abruptly by crisis, but gradually after several days: further, when there occurred the apparent relapse seen in the Chart, a sufficient explanation appeared in the renewed local lesions and general failing state of the animal, the character of the symptoms being 'septic,' and organisms in the blood not then multiplying. Such was my own interpretation of the phenomena, but it should be noted that with subsidence of the fever the spirilla did decline, their place in the blood being then taken by minute granular particles and filaments; and also that soon after their reappearance the health again deteriorated and fever returned. The entire history was as follows:—

The rat had been brought from an unhealthy quarter of the town, alone, and it had then a slight swelling on the back of the left hind foot, with œdema, being otherwise vigorous and active, and grunting loudly when approached,—e. t. 101·2°: it remained alive under observation for 38 days, during which period the blood was frequently drawn by puncture and the rectal temperature taken: in its capture it had probably been injured, and though seeming to recover somewhat, yet like other rats kept in similar constraint it did not thrive, being also necessarily much harassed by frequent manipulations. Death occurred suddenly at last, and at autopsy the blood was set, a peculiar sour smell

proceeding from the body: there was found serous inflammation of the pericardium and pleuræ, pyæmic infarcts in both lungs, most on right side with consolidation; liver large, pallid and infarcted with ova and a hydatid cyst; spleen large and pale, so the kidneys; the intestinal canal thin-walled; Peyerian glands unchanged, no entozoa here; blood from r. ventricle seemingly free of organisms; micrococci in the lymph and in the lung-nodules examined: in the intestinal mucus bacteria and ova of two kinds; no monads or spirilla seen: none of these data are peculiar.

Lastly, as to infectiveness of this spirillar blood-contamination. Soon after its capture (see the Chart) a drop of the infected blood of the rat was inoculated subcutaneously in a healthy rat (*a*) and in a healthy monkey, Macacus (*b*), whose temperature and blood were examined twice daily for six days continuously, with subsequent watching. The result of both these trials was negative: the monkey remaining well, and the inoculated rat only later becoming affected with gangrene of the hinder limbs (injured) such as other non-inoculated rats were liable to: its blood was found free from organisms the day before its death. Hence the inference that this particular form of blood-parasitism is not easily transmissible to the same healthy species, or to another animal—the monkey—known to be readily infected by similar inoculation of the human spirillar blood-contamination: all the data, therefore, as they stand, concurring to indicate a difference which is not without a certain significance.

# On the lately demonstrated Blood-contamination and Infective Disease of the Rat and of Equines in India.

BY

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(*With Plate V.*)

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In July 1877 the late lamented T. R. Lewis, soon after visiting Bombay to see the then newly-found relapsing fever organism of man—Obermeier's blood spirillum—made the observation, also novel here, that the blood of seemingly healthy rats often contains a ' flagellate organism ' which at first sight was not dissimilar to a spirillum. Towards the close of 1880 Dr. Griffith Evans, A.V.D., whilst investigating in the Punjab frontier the equine disease known as ' surra ' (a wasting disorder), made the further discovery that the blood of affected horses contained a specific parasite, which Dr. Lewis then identified as being very closely allied to the flagellated parasite of the rat; and by experiment Dr. Evans showed that the ' surra ' contamination and disease were both communicable through inoculation to healthy horses and also to the dog. In January 1885, Mr. J. H. Steel, A.V.D., when investigating a hitherto unrecognised disease amongst the transport mules in British Burma, found it to be the same as the ' surra ' of the N.-W. Provinces, and was further led to regard the specific organism as a ' spirilloid ' and the malady as a fever of relapsing type. Mr. Steel also showed that the monkey was susceptible of this infection, and at Dr. Evans' suggestion having applied to me for some technical information, he was good enough to send from Burma to Bombay several specimens of the tainted blood of mule, dog, and monkey, through means of which, by direct comparison, I too was enabled to identify the hæmatozoon of ' surra ' with that of healthy-looking rats ; and the subject being an interesting one, I continued to pursue it at leisure during 1885. My notes at that time were generally confirmative of prior morphological observations, including Lewis' latest published memorandum in the Quarterly Journal of Microscopical Science, July 1884 (which I had not then seen in India) ; and they also contain figures anticipating some quite recently given by Dr. E. M. Crookshank (Journal of the R. Microscopical Society, under date November 1886), who, in England, having examined Dr. Evans' specimens of the equine ' surra ' blood, and of the tainted rat's blood procured in London, considers the hæmatozoon in both to be identical in character,

and in more detail to belong to the genus *Trichomonas* of Leukart. It therefore appears that not only is the morbid equine hæmatozoon of the Punjab the same as that of Burma (Poona in the Deccan may now be added according to Mr. Steel), but also that both of them correspond to the hæmatozoon of rats at large in Calcutta and Bombay, and, moreover, to a like organism common in London rats. I will only add here that in India the camel may spontaneously suffer along with horses and mules; also that in Europe, with rats, the hamster may be thus contaminated, and certain fishes (the mud-fish and carp) with organisms not unlike.

*Data from Bombay.*—These include observations and experiments made with fresh material derived from rodents (*a*), and a short account of Mr. Steel's equine and other specimens from Burma (*b*).

(*a*) *Observations on rat's blood.*—Animals obtained were chiefly of the species *Mus decumanus* and *M. rattus* (?), the small brown or grey rat being oftenest found infected. A few examples examined of *M. bandicottus* and of *Sorex* sp. (also of mice) gave negative results. Males were the most numerous; in pregnant females the fœtus was not implicated: the animals were active, non-febrile, and, barring injuries incidental to capture, all seemed alike healthy, so that one could not surely guess beforehand the state of their blood. Material for scrutiny was taken from root of tail or from the paws by puncture after cleaning: only a small-sized drop could be had daily, and the admixture of some impurities was unavoidable by this method. For inoculation and peritoneal injections blood from the heart was used. A magnifying power of 300 linear commonly sufficed, but the higher powers were used and repeated scrutinies made when doubt existed; on such rarer occasions I have detected the Trichomonas in sealed specimens only on later trials, the number present being then extremely few. The animals were kept usually apart and fed on wheaten bread, rice, and water *ad libitum*: most were killed after examination, others preserved might survive for weeks or over a month, but most of them died at a much earlier period, apparently from the effects of confinement or possibly as the result of injuries received. The temperature was taken twice or thrice daily *per rectum*, and usually in control-animals as well as those under experiment. From 99°F. A.M. to 101° P.M. (37·3°—38·4°C.) seemed to be the common range under conditions stated; but degrees somewhat higher and lower were occasionally noted, attributable perhaps to excitement or exhaustion.

Of 60 rats caught in February, 9 (or 15 per cent.) showed the Trichomonas; their source was various rooms in a large hotel, and the infected commonly came in groups of two or three from one room, which might be on an upper floor. Of 150 rats caught in August to December, only 16 (barely 10 per cent.) were found infected; their source was, however, more varied, namely, from dwellings or warehouses in several parts of the native town, and as only individuals were

often brought the evidence of an endemic source of infection was here less apparent, and valid inference regarding a possible seasonal influence could not be made. At the hotel alluded to the rats had finally become too wary to enter the traps, but some infected animals from the kitchens were caught together. Inclusive proportion showing the hæmatozoon nearly 12 per cent., which is less than one-half of the percentage found in Calcutta and London.

A scrutiny of selected bi-daily records for 65 days did not reveal any pronounced or constant differences in the rectal temperature of infected rats as compared with the non-infected. And when it happened that the monads disappeared suddenly from the blood, there was never noted any striking corresponding change of temperature; indeed, as the Charts I have show, no change at all might ensue surpassing the normal range so far as known. Possibly under greater stringency some relationship of body-heat and this parasitism of the blood may be revealed, but none such has yet come to light.

I also failed to perceive any marked difference in the general state of the two sets of animals compared; both being equally liable to inconveniences or incidental hurt, either flourished or did not thrive in similar degree, for periods of several days or some weeks. If these data were not very numerous or rigidly exact, it was still clearly ascertained that the aspect of complete health was compatible with the presence of numerous flagellate organisms in the circulation. There was no evidence of a contagious influence.

A common lesion amongst animals kept was atrophy or necrosis of the tail, then usually the feet became swollen, and finally there were signs of paraplegia and extreme debility. No animal kept in confinement lived longer than six weeks, and the majority not more than a fortnight; death usually supervening gradually as if from exhaustion, and a terminal febrile state never being witnessed. Mere confinement seemed prejudicial; and even if the diet were unsuitable, the signs of actual starvation were not noted. Occasionally death was sudden and unsuspected, during the night, and as if spontaneous. All these events were common to infected and non-infected animals, about equally.

Fifteen special autopsies were made, of which 9 of rats showing the hæmatozoon, and in none of them did I see any peculiar coarse lesion of blood, or of viscera or tissues of the trunk. The brain was not examined. Microscopically, the only changes noted was an increase of leucocytes; the spleen elements seemed normal. Some details are the following, which may be useful to future observers:—

*No. 1.*—A large and active rat with swarms of monads in the blood: strangled and forthwith opened. The blood reddens and quickly sets; no marked changes anywhere visible: liver-tissue healthy and free from ova or hydatid. No intestinal entozoa, some hyperæmia of mucosa in ileum and clear mucus near the ileocæcal valve: the mucus of duodenum and ileum free from monads. Cœcum filled with greenish soft pulp containing



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many large ovoid ciliated monads (? Trichomonas) differing from the commoner form (Cercomonas). Larger infusorial species have been found in the cœcum.

*No. 2.*—A small animal, with parasites being much injured, was killed. Organs of chest and abdomen of healthy aspect. The intestines contained a greenish pulpy food-mass and were wholly free of entozoa or ova, nor were monads found in mucus of duodenum, jejunum, or ileum.

*No. 3.*—A small rat sickened and died, with hæmatozoa to the last. Body emaciated, heart and lungs of normal aspect, a small hydatid in the liver, no ova. The spleen elements normal : kidneys healthy-looking : stomach and intestines thin-walled, thin fæces present, a single worm (Echinorhynchus) in the small gut. No monads in the duodenal mucus.

In the next three animals the hæmatozoon had disappeared shortly before decease.

*No. 4.*—Pregnant female, not injured, non-febrile ; became weak and died 6 days after capture, with sores ; no visible cause of death. Parasites in liver and intestines as found in non-infected rats. The blood of two fœtuses free from hæmatozoa.

*No. 5.*—Died 7 days after disappearance of the organisms. Aspect of blood not peculiar ; coagulation and rigor mortis quick : viscera normal ; ova in the liver ; spleen unchanged : the feet œdematous, no bacilli in the serum there.

*No. 6.*—Died 7 days after the gradual cessation of blood-infection. Emaciated, tail necrosed ; at autopsy only wasting of the viscera.

Amongst rats always free from blood-contamination, so far as known, there was one autopsy with intestinal entozoa and ova in the liver as in No. 4 above : and another with the liver crammed with ova and no entozoa, the blood charged with leucocytes and plasm-clumps : in two other examples ova in the liver and numerous monads in the intestinal mucus.

Surgeon-Major Lewis' account of the rat hæmatozoon being easily accessible, it is not needful for me to offer a new description of it. Dr. Crookshank has added two other characters, namely, a spine-like process at the hinder thicker extremity and a lateral longitudinal membrane ; remarking also that the flagellum pertains to the front or advancing end of the organism, thus as in the infusoria generally acting as a tractellum and not as a propelling means or pulsellum. These features I have been able to see and confirm.

The hæmatozoon should be examined not only in its fresh active state but as well after exposure to the fumes of osmic acid (which kills without distorting), and after dessication and staining as done in Bacterial research : for each method furnishes useful data.

When, as usual, tolerably numerous in the layer of fresh-drawn blood, the parasites seem to be diffused and are readily detectible through means of the peculiar twirling motion of the red discs in their proximity ; when but few exist, a patient or repeated scrutiny of the blood is needed ; the aspects are the same when few or many are present. As the material of those organisms, like that of bacteria, resists the action of acids and alkalies sufficiently concentrated to destroy obscuring red discs, a measurement of their total number is best made after drying the specimens of blood, and before staining exposing them

to the action of acetic acid or liquor potassæ; all the filaments present, by this method, becoming visible and readily counted. Often the number present in the field seems equal to or greater than that of the leucocytes, and occasionally comparable to a percentage of the red discs themselves; such a swarming may, however, be illusive, yet if the parasites were only $\frac{1}{500}$ the number of red discs, their total in the circulation would amount to some millions. As to the movements exhibited, whilst it is possible such may be exaggerated outside the body under conditions abnormal and likely to prove irritative, still it may be assumed that during their stay within the blood-vessels, these organisms maintain a degree of activity curious to contemplate. As seen in the freshest blood, their movements are so rapid as to be followed with difficulty by the eye, certain optical impediments concurring thereto; yet evidently the chief one is that of progression more or less direct, and accompanied by a lateral lashing or sometimes an apparent rotation. Progression may doubtless be either backward or forward, and misled by the idea of the thicker end being the head of the organism, I (like Lewis) gained the impression that it commonly advanced; yet it is certain that when more tranquilly performed, progression is really with the flagellate extremity in front, as is usual in the other infusorial monads: this datum is important.

In the fresh state, the substance of these organisms appeared under the higher powers to be homogeneous and translucent; yet, as described below, there presently appeared one or more bright spots in the thicker part. The very delicate lateral membrane became visible only in my osmic acid and stained specimens, and these serve to conform Dr. Crookshank's later observations to a considerable extent (see the figures in Plate V.) Aniline staining, which has proved so serviceable in Bacterial researches, did not seem to me equally trustworthy here, on account of the inconstant degree and site of colouring which it imparted; at least by this means I did not become assured of the presence of internal organs, or of definite series of reproductive particles. Mr. Steel's specimens from Rangoon however, when stained with methyl-violet, showed distinct appearances of immature organisms or rudiments, such as I find Dr. Crookshank has also detected and figured in the rat's blood: they merit further attention.

The next topic I would notice is the changes exhibited by these hæmotozoa in blood hermetically sealed, at rest and at a temperature of 70°—80°F. in the shade. Numerous experiments were made in February and in September 1885, and at the time I was not aware that Lewis had made similar observations as recorded in his second Memoir: subsequently Dr. Crookshank has also alluded to this subject. My own results may be illustrated by the following extracts from Note-books preserved:—

*February.*—A minute clot of infected rat's blood was covered with a drop of ·75 per cent. solution of common salt, and examined at intervals with $\frac{1}{10}$ inch water immersion lens.

After 3 hours, in the lateral open spaces movement less active, the thick end of the filaments seems to drag and bulge at intervals, showing a contractility of its substance which is still uniformly clear: blood-cells unchanged, the red forming rouleaux; some micrococci have appeared oscillating apart. As the monads become quiscent they seem more slender and flattened, the ribbon-like margin wavy or angular; and that this change is not due to simple dessication is evident from the red discs preserving their form and floating freely: next, a stiff crumpled aspect ensues, the body of the organism assuming a skate-like form, the flagellum being still round, flexible, and mobile. At 4½ hours a few bright dots appear in the motionless body, the flagellum still occasionally lashing spasmodically as it were and now it would be hard to recognise the monad. At 5 hours, the bright dots are distinct but not more numerous, the flagellum bent, clear and quiet: red discs unaltered. Another specimen shows that the bright specks might be due mainly to knots or foldings, but some resemble 'vacuoles.' Not every one of the organisms here presents identical changes at the same hour, nor are all the red discs precisely alike; therefore some difference of individual properties may be inferred. The above description applies to the parts towards the edge of the thin glass cover, whilst nearer the centre of the preparation where the blood-clot rests the changes are very much less marked and the organisms are still active; in the central part, appearances remain precisely as at beginning of the experiment. Shortly afterwards, the changed monads at the sides were but faintly visible; and on the following day (27 hours) no other changes indicative of development were to be seen in the specimens, which had then become clouded. In an experiment with blood diluted with aqueous humour of the rat, the monads had in the course of 4 hours become shrunken, bent, stiff, granular, and motionless: whilst in similar blood diluted with the salt-solution, they remained very active at a like interval.

*September.*—Further trials made, and as a sample the following is quoted:—After 22 hours the organisms in the centre of the preparation grouped in open spaces mostly quiescent; the thicker end is pointed and the body shows a clear space between two bright spots; towards the margin they were more shrivelled, granular, and dotted in their whole length, excepting the flagellum itself. Red blood-discs somewhat mis-shapen, leucocytes, quiescent, and filled with granules; a few free cocci present, no bacilli. A moving monad in the centre being watched, it advanced like a worm, the attenuated end in front, and the hinder thicker end being drawn up towards the front end fixed as a kind of fulcrum: the first stage in this process is the projection of the flagellum; and the next the dragging forward of the thicker part by successive wriggling movements not wholly sluggish: the body or thick part is distinctly granular, and contains bright particles of different dimensions and seemingly imbedded in its substance. Occasionally a brief retrograde movement is noticed. Later (24 hours), the body sometimes is flattened, angular, dotted, and quiescent, the flagellum still being jerked at brief intervals; it seems as if the body becomes too heavy, or is the first to fail; one or two of the bright granules are largest and connected with the angular projections. Micrococci free are present, and others apparently growing within the leucocytes. At 46 hours the monads not much altered in the centre of the preparation; the broader part is quiescent, expanded, angular, and of granular aspect; the narrow end is distinct and languidly moves at intervals. The blood-elements are hardly altered: active micrococci are seen in some places. At 76 hours all is faint yet clear, the blood-cells not greatly changed; the monads are still visible, though shrunken, granular and bent, with no signs of growth. At 117 hours the organisms visible, with granules in their thicker part, but no signs of spore-production, the attenuated end is clear and possibly a little swollen: red blood-discs faint, leucocytes

distinct with their granular contents. At 141 hours aspects the same, apparently some diminution of the granules in the body of the monads, with a clearness like those within the leucocytes present. Twenty-four hours later no change except that the parts are fainter. (*See* Plate V, fig. 6.)

From my observations it might be inferred that at ordinary air-temperatures, in the absence of a continuous supply of oxygen and nutriment, these flagellated organisms do not grow in the blood: they do not, however, immediately dissolve and disappear, and the early drifting away from the centre, rigidity and quiescence, are phenomena which I know to occur with the febrile blood-spirillum of man prior to and consentaneous with a very decided growth in bulk. As to the significance of the shortening and dotting of the bodies of these monads, under the circumstances named, the alternative inferences of an incipient spore-production or of a mere plasmic degeneration would probably be decided in favour of the view of decay; yet this seems to be not absolutely certain.

*Inoculation experiments.*—These were as follows:—the subcutaneous injection of healthy animals with tainted blood of the rat, the animals so employed being the rat, dog, cat, horse, and monkey; also the intra-peritoneal injection of a healthy rabbit and a healthy monkey. The last-named attempt proved alone successful, but it may be useful to offer a brief record of the whole series of trials.

(*a*) EXPERIMENTS ON RATS:—1 and 2. A fragment $\frac{1}{10}$ square inch large of fresh blood-clot was introduced beneath the skin of the thigh of two animals with seemingly healthy blood. Second day, the blood of both preserves its normal aspect: so on the third, fourth, fifth, sixth, and seventh day. One rat now escaped; the other continued well and was submitted to observation daily until the sixteenth day, when, the result being so far negative, it was killed and inspected without the detection of special morbid change. 3. A similar fragment of blood-clot from the heart of rat which had just died with flagellate organisms still persisting in the circulation was introduced beneath the skin of the thigh of an animal with healthy-looking blood. Evening temperature 101°; the animal looks well: second day, morning temperature 101·8°; the blood unchanged; e.t. 102·4°, no change and no evident constitutional irritation with this rise: third day m.t. 101·8°, *nil*, e.t. 102·4°, *nil:* fourth day m.t. 100·6°, *nil*, e.t. 101·6°, *nil:* fifth day m.t. 100·4°, *nil*, e.t. 101°, *nil:* sixth day m.t. 100 4°, *nil*, e.t. 101·4°, *nil:* seventh day m.t. 100°, *nil*, e.t. 101·6°, *nil:* eighth day m.t. 100°, *nil*, e.t 101·6°, *nil:* ninth day m.t. 99·8°, e.t. 101·2°: tenth day m.t. 100°, *nil*, e.t. 100°: eleventh day m.t. 100°, *nil*, e.t. 101·2°; and the animal continued well for five days longer.

(*b*) EXPERIMENTS ON THE DOG:—1. A healthy white puppy, a month old, had a few minims of fresh rat's blood containing several monads injected in the thigh: e.t. 100 6°; no irritation of wound: second day m.t. 100·6°; blood unchanged; e.t. 101°, *nil:* third day m.t. 100°, e.t. 101·2°, *nil:* fourth day m.t. 99°, *nil*, e.t. 100·6°: fifth day m.t. 100·4°, *nil*, e.t. 101°: sixth day m.t. 99·6° and e.t. 100°; *nil*, and so on for five days longer. 2. A healthy black male pup was injected with similar blood; e.t. 101·4°; nothing morbid visible in its blood: second day m.t. 101°, *nil*, e.t. 100°, *nil:* third day m.t. 99°, e.t. 101·4°, *nil:* fourth day m.t. 100°, *nil*, e.t. 101°: fifth day m.t. 101·4°, *nil*, e.t. 100·4°: sixth day m.t. 100° and e.t. 100·4°, *nil*, and so on for six days longer.

(*c*) EXPERIMENTS ON THE CAT :—1. A large black healthy male ; e.t. 101°; had inoculated at inner surface of thigh a minute clot of rat's blood filled with active organisms : second day m.t. 98° ; was again inoculated with a small clot from another rat just killed, which contained many monads,—this being done to ensure thorough trial ; e.t. 100° *nil* : third day m.t. 98°, blood *nil*, the wounds scarred ; e.t. 102°; animal feverish, but eats heartily and sites of inoculation not inflamed ; a few large granule-cells observed in the blood : fourth day m.t. 98° ; nothing abnormal seen in the blood except a few large granule-cells ; e.t. 101·6°; nothing abnormal in the blood but some large plasm-particles and a few large granule-cells : fifth day m.t. 99°, *nil*, e.t. 101°, *nil* : sixth day m.t. 98°, *nil*, e.t. 101°, *nil* : seventh day m.t. 99°o, *nil*, e.t. 99°: eighth day m.t. 100°: *nil*, e t. 101·6°: ninth day m.t. 99° and e.t. 100·6° *nil* : for five days longer the temperature remained normal. 2. A healthy fawn-coloured small cat; e.t. 101·4°, had inoculated in the thigh on point of scalpel a minute clot of blood from a rat which contained the monads : second day m.t. 99°; blood unchanged ; e.t. 100·2°; white cells many in the blood, which seemed poor in quality : third day m.t. 99°, *nil*, e.t. 100·6°, *nil*, white cells many : fourth day m.t. 99° ; only leucocytes and small plasm-particles unusual ; e.t. 101·6°; leucocytes and large plasm-particles : fifth day m.t. *nil*, blood healthier looking ; e.t. 99°, *nil* : sixth day m.t. 99°, *nil*, e.t. 101·6°, *nil* : seventh day m.t. 98°, e.t. 103°; animal feverish, in its blood nothing unusual, but many white cells and no other apparent cause of the fever : eighth day m.t. 100·4° *nil*, e.t. 100·6°: ninth day m.t. 100·4° and e.t. 101·4°, *nil* : for five days longer the temperature normal.

(*d*) EXPERIMENTS ON MONKEYS :—1. A small fresh-brought healthy animal ; had at 10 A.M. injected m. xx of infected blood of rat just before killed on each side of thighs ; temperature 102°: at 5 P.M.t. 103°; nothing abnormal seen in the blood; wounds unchanged; animal quiet : second day m.t. 100·4°; nothing abnormal in the blood ; e.t. 101·6°, *nil* : third day m.t. 101° and e t. 102°; no visible change in blood, but animal seems oppressed : fourth day m.t. 100°, *nil*, e.t. 101·4°, *nil* : fifth day m.t. 100·6°; nothing unusual in the blood, but some granule-cells ; e.t. 101·8°; nothing but some plasmic particles : sixth day m.t. 100·4°, *nil*, e.t. 100·4°, *nil* : seventh day m.t. 100°, *nil*, e.t. 101·8° *nil* : eighth day m.t. 99° and e.t. 101°: ninth day m.t. 100°, *nil*, e t. 101° : tenth day m.t. 100·4° and e.t. 101° *nil* : and for five days more temperature remained normal.

2. A larger monkey, healthy as regards temperature and blood ; had injected m. xx of fresh-drawn blood of rat containing many monads ; e.t. 101·6°; its blood unchanged : second day m.t. 99°, *nil*, e.t. 101°, *nil* : third day m.t. 99°, *nil*, e.t. 101·6°, *nil* : fourth day m.t. 100°, e.t. 101°, *nil* : fifth day m.t. 100° *nil*, e.t. 101 6°, *nil* : seventh day m.t. 100°, *nil*, e.t. 100·6°: eighth day m.t. 99·4° and e.t. 100° *nil*; for five days longer a normal temperature.

These observations were made by myself in the month of February.

(*e*) EXPERIMENTS ON EQUINES :—These I was unable to carry out personally, but considering it particularly desirable to ascertain if the seemingly harmless rat-infection could be communicated to the horse, in which animal an apparently similar parasitism of the blood is attended with the symptoms of serious illness, I forwarded on different occasions, towards the close of 1885, several Bombay rats, having the hæmatozoon in their blood, to my friend Mr. J. H. Steel, A.V.D., then at Poona in the Deccan, with a request that he would be good enough to inoculate horses, as occasion offered, with the blood of these rats in the same manner as had been successfully done in Burma with the blood of equines suffering from the ' surra ' disease. And in January 1886 I received an interesting record, from which the following extracts are made :—

(1) At mid-day infected rat's blood mixed with water was injected subcutaneously in the region of the neck and also into the peritoneal cavity of a horse ; about

80 minims being injected at each site : at 2-20 P.M. temperature 101·9°, and no organism detectible in the horse's blood : next day there was observed a diffuse painful hard swelling at both sites of injection, and the patient seemed a little feverish and excitable : these symptoms subsided and the animal was subsequently lost sight of.

(2) An aged horse had inoculated subcutaneously some infected rat's blood mixed with water : tumefaction occurred at the site of inoculation and no other result is mentioned

(3) The blood of a rat mingled with luke-warm water was injected subcutaneously in a mare and a pony. The mare received 80 minims in front of the right shoulder : on the following day marked swelling was perceptible at the spot, which however speedily declined until hardly perceptible on the ninth day : slight irregularities of the temperature were noted on two occasions, and up to the nineteenth morning, when observation ceased, no spirilloid could be found in the mare's blood, which had been daily inspected : some weeks later the animal continued in good health. The pony received at several punctures in the neck altogether about 90 minims of mixed rat-blood and water : and for the nineteen days ensuing there were noticed only some slight perturbations of temperature, slight swelling at the sites of inoculation subsiding on the fourth day, and an absence on each day of any visible contamination of the blood of the pony, which some weeks later continued in good health.

(4) A chesnut pony mare in good condition received in each jugular vein about 65 minims of blood from a rat mixed with tepid water—this blood teeming with very active organisms: a smart rise of temperature occurred seven hours afterwards; there was no local swelling, and no parasite could be detected in the blood of the pony on the first, second, third, fourth, and sixth day of experiment, nor at a later date was any positive result communicated.

Regarding these trials ending thus negatively, Mr. Steel remarks that "although the amount of rat's blood used in each case has been very small, it is to be remembered that an equal amount of blood from an infected mule would convey the 'surra' disease to a healthy mule by either of the methods of administration adopted."

(*f*) In September 1885 I made the following essays of intra-peritoneal inoculation, the subcutaneous method having hitherto failed. Rat's blood containing numerous monads promptly taken from the heart and diluted with an equal part of warmed salt-solution was introduced by syringe to the amount of 5 minims into the peritoneal cavity of a rabbit and a monkey whose blood was previously ascertained to present normal aspect.

(1) Rabbit, young; e.t. 101·6°: second day m.t. 100·4°; no change in the blood twice examined ; e.t. 102·2°; animal seems irritated, its blood unchanged : third day m.t. 100·4°, *nil*, e.t. 102 6°, *nil :* fourth day m.t. 101·4°, *nil*, e.t. 101°, *nil*, fibrine and white cells scanty : fifth day m.t. 100°, *nil*, e.t. 101·4° *nil :* sixth day m. t. 100° *nil*, e. t. 10° *nil :* animal lively : seventh day m. t. 101° *nil*, e. t. 102°: eighth day 100·6° *nil*, e.t. 101·8°: ninth day m.t. 100·4° *nil*, e.t. 101·6°: tenth day m.t. 100·4° *nil*, e.t. 100·4° : eleventh day m.t. 100·4° *nil*, e.t. 101·4°: twelfth day m.t. 100·2° *nil*, e.t. 101°: thirteenth day m.t. 100·4°: *nil*, e.t. 101·6°: fourteenth day m.t. 100° *nil*, e.t. 101°: fifteenth day m.t.

99·8°, *nil*, e. t. 100°: sixteenth day m. t. 99·6°, *nil;* and for a few days longer similar negative results.

(2) A small monkey (*Macacus radiatus*) with healthy aspect and blood; m. t. 101° possibly not all the injected material reached the peritoneal cavity, as the blood had congulated and it may have been blood-serum chiefly which penetrated deeply. Second day m. t. 100°; 18 hours after experiment the animal seems well, no local swelling, but abdomen slightly tender at site of puncture to the right of the navel; in the first sample of blood taken from a digit 2 monads were detected very actively moving and otherwise presenting all the aspects of the hæmatozoon seen the day previously in the rat; they kept near to each other and were long watched: temperature at 1° P.M. was 100·6°; at 5 P.M. t. 102°; another active organism was seen in a fresh specimen of the blood from another digit: t. at 9° P.M. 100°: third day m. t. 101°; a specimen of the blood now taken showed a single active monad: at 1° P.M. t. 100°6; at 5 P.M. t. 102°; a flagellate organism like that of the rat was again found in the blood, as before: at 9° P.M. t. 100·2°: fourth day m. t. 101·4°; I failed to detect any parasite; e. t. 101·6°, ; no parasite seen, but much plasmic matter ? and many white cells; at 9° P.M. t. 99·6°: fifth day m.t. 100·4° *nil*, e.t. 102°; plasmic particles small, many: at 9° P.M. t. 100°: sixth day m.t. 100·6° *nil.*, at 1° P.M. 100·4°, at 5 P.M. t. 101°, *nil*, at 9° P.M. t. 100°: seventh day m.t. 100·4°, *nil*, e.t. 101·6° and later 99·6°: eighth day m.t. 100·2°, *nil*, but many plasmic particles, e.t. 101·4° and 99°: ninth day m.t. 100·4° *nil.* e.t. 101·4° and 99·6°: tenth day m.t. 100·2°, *nil*, e.t. 102°, *nil*, at. 9° P.M. t. 100°: eleventh day m.t. 100·8°, e.t.s. 101·6° and 99·6°: twelfth day m.t. 100·4°, *nil*, e.t.s. 101·6° and 99·4°: thirteenth day m.t. 100·4° *nil*, e.t. 102° *nil;* at 9° P.M. t. 100°: fourteenth day m.t. 101° *nil*, e.t.s, 102° and 99·6°: fifteenth day m.t. 100·4° *nil*, e.t.s. 101·6° and 99·8°: sixteenth day m.t. 100·4° *nil*, e.t.s. 101·6° and 100°: seventeenth day m.t. 100·4° *nil;* and subsequently this animal long remained well.

SUMMARY OF EXPERIMENTAL RESULTS:—Of 15 varied trials one furnished decided positive results, and the chief object I had in view was thus attained. Records are detailed here for the guidance of future investigators; and the conditions of effective inoculation being probably complex, some amendments in the methods above used will doubtless suggest themselves. Culture of the inoculated blood seems a promising means, varied at discretion. Respecting the successful result in the monkey, to all appearance the organism newly visible in the blood was identical with those before found in the rat; and I calculated that if there were no more than 3 ounces of blood in the monkey and only 2 monads in a drop, there might have been about 3,000 of these organisms circulating on the second day after inoculation, with a probability of the total being really larger. It did not seem quite certain that a reproduction of the rat's organism had taken place in the circulation of the monkey; but possibly this was so to a very limited extent. By experiment it is known that on mixing tainted blood with healthy mule's blood, or that of healthy bullock or goat, the organisms are not at once rendered quiescent, or killed. (Steel.)

Having now given the data acquired in Bombay, I proceed to compare them briefly with other information, including the material sent to me by Mr. Steel from British Burma.

For valid inference it was obviously needful to ascertain the sameness of the things under comparison. This might be done either morphologically or biologically; and one striking feature of the present instance consists in its offering an apparent discrepancy in these two respects, which, so far as I know, is unique in the annals of experimental pathology.

First, as to the morphology of the hæmatozoon. Judging from description, there can be no doubt that the organism found in Bombay is identical with that detected by Lewis in Bengal; such inference being confirmed by the micro-photographs appended to the Calcutta Memoir of 1879 and the remarks and drawings in Dr. Lewis' "Further Observations," London, 1884. Here I may note also the very close similarity (at least) of the Indian hæmatozoon with that of the German "hamster" described by Wittick and illustrated by excellent photographs, with a comment in Koch's well-known article Mittheill. a. d. Konigl. Gesund. amt, Band. 1—Berlin, 1881. And not less evident is the identity therewith of the London flagellate protozoa more recently described and illustrated by Dr. E. M. Crookshank l. c. 1886. I am not acquainted with other details ( if any ) published in India or elsewhere respecting the seemingly non-pathogenic hæmatozoon of rodents; but the above concurrent testimony is sufficient to establish the wide prevalence, as well as identity, of this species of blood-parasitism.

Adverting, next, to the pathogenous equine-form, my observations date from the beginning of 1885, on the receipt from Rangoon of Mr. Steel's dried specimens of blood of the naturally diseased mule, and the artificially infected mule, dog and monkey, taken at different periods of illness; the preparations arrived at Bombay in fair condition and were successfully stained with methylene blue, methyl-violet, and fuchsine. After careful and repeated scrutinies, I arrived at the conviction that no essential difference in aspect existed between the organisms present in the natural and the acquired disease, nor between either of these sets and the common rat-infection. Learning subsequently that Dr. Lewis had arrived at a similar conclusion, as regards the hæmatozoon of Calcutta rats and of the Punjab "surra" mule disease, as well as of a dog infected therefrom, my notes were allowed to stand over. Dr. Crookshank has since demonstrated that the organism from the Punjab entirely corresponds in aspect to that found in London rats. Lastly, Mr. Steel, having become well acquainted with the Bombay organism in the fresh state, is also of opinion that their aspect is identical with that of the parasites in fresh "surra" blood as seen in Burma. More recently still the equine malady and its hæmatozoon have been detected by the same observer at Poona in the Deccan; so that India is the seat of a widespread and very serious equine-disease, having for one of its characteristics a blood-contamination seemingly identical with one largely prevalent amongst common rodents of the country. Other countries may be in the same predicament.

The several links in the above chain of identifications seem nearly equally strong and connected; for observers have been competent, and in itself the hæmatozoon is peculiar enough to be readily recognised, having in each of the instances quoted preserved a similarity of form, dimensions, structure and movements which seems never to have surpassed the limits of a possible normal or incidental variation. In both rodent and equines the numbers visible at a time, their relation to the ordinary blood elements, changes on keeping and behaviour with re-agents, have been also practically identical; the entire evidence being as valid as that commonly accepted when widely-separated observations are inter-compared. I doubt, indeed, if by simple inspection the infected rat's blood could be distinguished certainly from that of the "surra" mule, dog, or monkey. This is not to affirm that no visible differences actually exist, but that in each instance characteristics have been maintained amidst differences not more frequent or considerable than might be anticipated from the varying conditions of observation.

Secondly, as to biological aspects. The vital properties of the blood-parasite as concerns the organism itself will be alluded to under the next heading; and respecting its influence upon the state of the infected hosts, both rodent and equine, the following remarks are offered. As regards rats, the testimony of Lewis and Crookshank has been concurrent with my own experience narrated above, being to the effect that the health of the infested rodent does not seem impaired either generally or specially, as contrasted with that of other captured rats whose blood was free from visible contamination. I further ascertained that no alterations of temperature occurred with the presence, sudden disappearance, and absence of the hæmatozoon. Apart from the general nutrition, no special function seemed disturbed; and at autopsies no particular organic lesion was noted. When infested rats died spontaneously, it was not differently or more obscurely than happened in the case of the non-infected similarly deceasing. On the other hand, the entire history of any individual not being known, it was not certain that a true normal standard of comparison had been available: moreover, the first entry of the parasites into the blood was never witnessed, and the influence of repeated infection was not learnt; in such small animals blood-observations were necessarily limited in number and volume, and much may have been overlooked: moreover, as to sickness and decease, the inevitable daily manipulations and punctures, added to injuries inflicted during capture, constraint, and change of diet, would of themselves cause a disturbance of the system possibly interfering with a correct diagnosis; and, in sum, I was not disposed to regard my own results as final.

In his comment on the infested hamster, Dr. Koch, noting the occurrence of spontaneous sickness and death, with on autopsy no lesion in explanation, insists rather upon the contaminated state of the blood; nothing, however, is

said of comparison with non-infected animals dying in confinement, or of experimental inoculations. Results being so far negative, there is next to state that mere obscurity of symptoms and lesion does not preclude the possible existence of a veritable blood-poisoning; the biological proof of which would be the artificial infection of a healthy animal of the same or near species. As regards healthy rats, according to my experiments these did not suffer by inoculation with the tainted rat-blood hypodermically made; nor did cats, dogs, or monkeys; nor did the rabbit inoculated through the peritoneal sac; nor the horse as tested by Mr. Steel in both these methods and also by intra-venous injections. In not one of these instances was there any communication to the healthy animal of either visible or occult blood-contamination, as proved by the unaffected general state of the recipient and by the absence in its blood of an organism like the rat's hæmatozoon. Here the last of my experiments on the monkey by intra-peritoneal injection possesses a special significance, since it demonstrated that the hæmatozoon which seems innocuous to the rat is also harmless to the monkey, so far as may be judged from a single datum carefully acquired.

Adverting now to the biological properties of the same blood-parasite as manifested amongst equine animals (also the camel), and in the dog and monkey when inoculated from equines, a notable difference becomes apparent. Thus it has been found by Dr. Evans and Mr. Steel (and since by Mr. Gunn, A.V.D. ) that in the horse and mule the presence of this hæmatozoon is constantly and exclusively accompanied by a serious general disorder which in its symptoms is more or less protracted and emaciating, at intervals febrile, icteric and hæmorrhagic, intractable by remedies, and in its termination fatal, without the production of any strictly pathognomonic lesion excepting that of the blood. Observers agree that this malady is not contagious or infectious in the ordinary sense: naturally occurring it is endemic, seasonal and limited, so far as appears, to the horse, mule, and camel; it may prevail, extensively, and has been popularly recognised by various names, of which "surra" is one, that of "equine relapsing fever" being a designation proposed by my friend Mr. Steel (see below). From the statements made it follows that the disease is a general one, not less characteristic in its symptoms than in its visible blood-contamination; and though not naturally communicated by contact or proximity, both these features may, I next note, be artificially reproduced by inoculation of the infected blood hypodermically and otherwise in other healthy animals. On this point the data are concisely as follows:—Dr. Evans' transmission of the disease to the horse by subcutaneous injection (2) and through the stomach (2), to the dog by subcutaneous injection (1) and through either mother's milk or stomach (1 experiment) : Mr. Steel's transmission to the mule (several times) and pony (1) subcutaneously and by stomach, to the dog subcutaneously (1) and to the monkey also (1). To this

list may be added inoculation of the dog (1) with reproduction of the hæmatozoon, by Mr. Gunn, A.V.D., (Quar. Jour. of Veter. Science in India, No. 19, page 240, April 1887). In most of these instances details of experiment are given sufficient to show that adequate precautions against error were duly practised : seemingly the results were never of a doubtful character ; and, lastly, it would appear that the inoculation experiments were invariably successful, so far as concerns repetition of the blood-organisms. As regards identity of the morbid state induced with that naturally acquired, not less ample proof is afforded in the instance of the horse and mule,—see Appendices D, E, F, and G of Dr. Evans' Report; and Mr. Steel, in reply to some questions of mine, explicitly states of his experiments that "the phenomena induced by inoculation were those of 'surra'" and "all inoculated animals died of 'surra' pure and simple, with the exception of one case in which pyæmic complications" ensued some days before death. According to these data, which, if not numerous, are still decided and clear, it would follow, first, that both visible blood-contamination and a certain group of symptoms are transmissible by artificial means from the diseased equine to healthy ones of the same species ; and, secondly, that a similarly contaminated state of the blood may be transmitted from the "surra" equine to the dog and monkey. That to these non-equine animals, however, the "surra" disease had always been simultaneously communicated, is not so clear, for the records bearing on this important point seem too few and incomplete to be quite convincing. Thus, while the two dogs inoculated by Dr. Evans both showed on the 11th day a kind of leukhæmia (and one the hæmatozoon scantily) with pallor and emaciation, yet there was no jaundice or petechial rash ; and, moreover, a sucking pup, which on several occasions displayed abundant blood-organisms, continued in good health for at least 18 days. The dog, too, inoculated by Mr. Gunn did not for days or probably weeks show any signs of illness whilst the blood contained active monads. Continuous records are, however, wanting, and the end was not known. The two positive data of Mr. Steel include, first, a young dog both inoculated and fed on tainted mule's flesh ; organisms seen in blood on 12th day, but no signs of general disorder until the 16th, when and after the animal was dull, feverish, with very pallid conjunctivæ, a slight cough and tenderness in the loins, appetite capricious, many parasites in the blood at times : 28 days later there were noticed fever, emaciation, with glandular swellings on each side of the head and in the left groin, with a state of general tenderness ; in the blood many disintegrated corpuscles, but no parasites : the buboes increased, œdema of the lower limbs, with persistent fever and tendency to collapse, death ensuing on the 51st day after inoculation. The autopsy was made too late for useful results. Next, a monkey had a syringeful of tainted mule's blood injected beneath the skin of the thigh : 3 days afterwards high fever and organisms in the blood, which lasted 4 days, subsiding on the 5th, but recurring

after an interval of 4 days; and subsequently other febrile recurrences (parasites seen after a month) with emaciation and debility, feebleness in the limbs, ulcers and necrosis of the bones of the feet, and final exhaustion upwards of two months after inoculation: at the autopsy no marked positive changes, only some petechiæ on surface of lungs. This animal was brought from Rangoon to Madras during its illness, and may have suffered, as monkeys often do, from confinement and the voyage: its temperature chart for 30 days is given by Mr. Steel, blood-scrutinies being made for 13 days in succession at first and again later on.

Some other inferences from the records, as they stand, are the following:—Incubation period after inoculation, varied as to the subject, being in the horse 4—6 days, in the mule 4—10 days, in the dog 11 or 12 days, in the monkey about 3 days; also varied as to the method of infection, being shortest after intra-venous injection and subcutaneous inoculation, and somewhat delayed after simple ingestion of the tainted blood given in quantity. Sometimes a depression of temperature occurred during this period. A local swelling might or might not follow the hypodermic operation. There was one successful infection of a mule with blood taken from a mule naturally diseased, but not at the time of experiment showing the parasite in its fresh state: organisms appeared in the subject on the 10th day, along with pyrexia high and intermitting, and subsequently death on the 40th day. Mr. Steel thinks it possible that in stained preparations of the inoculating material some evidence of the presence of the hæmatozoon might have been seen. Dr. Evans found that when the tainted horse-blood was freely diluted with water, the organisms had disappeared after 24 hours, and this mixture proved inocuous to two healthy horses when administered by mouth: and Mr. Steel ascertained that thoroughly-dried tainted mule's blood was inoperative on inoculation. A fatal result seems always to have ensued in watched animals,—amongst equines in 5—7 weeks: a dog lived 7½ weeks; a monkey over two months: the *post-mortem* appearances being occasionally recorded as not essentially peculiar. Second or successive series of inoculations from the first individual were not apparently attempted. Mr. Steel's essays include an unsuccessful administration of gastric and intestinal contents of tainted mule per mouth to a healthy animal. Also unsuccessful inoculation-experiments (three) on an Amrut bullock, old and emaciated: and similar unsuccessful inoculations on a young goat (three), the animal becoming, however, much emaciated, in spite of good feeding and voracious appetite, after the last essay.

The last part of my Notes refers to the probable nature and analogues of this blood-parasite and to the character and affinities of the equine "surra" disease.

As regards the hæmatozoon, minute elongated bodies, of simple homogeneous structure and provided with cilia or a flagellum, might, I suppose, be

regarded as either bacterial or infusorial forms, or less probably as zoospores or possibly as embryos of nemat helminths. Other analogues may exist.[1]

Comparison with the immature young of nematode worms is alluded to, because it happens that Dr. Evans once found numerous embryos of a filaria in the blood of a camel in bad health and associated with four other camels affected with genuine "surra;" and Mr. Steel also detected in the blood of a mule under experiment a nematode "worm;" the hæmatozoon under notice being, however, absent in both these instances. I never met with blood-filariæ in the rat, nor had Dr. Lewis. Micro-filariæ commonly present characters wholly diverse, being much larger in dimensions, cylindrical or vermiform, non-flagellate, of granular aspect or showing traces of internal organs, and sometimes enclosed within a delicate sheath: besides, the direction of movement is not uniform; and it has not been shown that these temporary tenants of the blood can be reproduced through inoculation.

As to comparison with spermatic elements, the late Dr. Lewis observed (in his second Memoir) that he was once inclined to think that the flagellated organisms of the rat might be the spermatozoa of some parasite hidden in the tissues of the animal; mentioning their amazing similarity to the spermatozoids of a Tænia seen in the intestines of rat, whose blood, however, was at the time free from organisms. The lateral membrane of the hæmatozoon would also call to mind the similar appendage of spermatic filaments perhaps elsewhere present than in certain lizards. But all such filaments move the thick end foremost, their flagellum being a propeller; and, besides, they are not independent organisms capable of reproduction *per se*.

The bacterial character of these blood-parasites is disproved by their non-cellular aspect, and by their contractile plasma in which rapid changes of length and thickness are seen wholly unknown amongst the algoid filaments: moreover, their prompt decay after a few hours' keeping, with entire absence of signs of reproduction by division or spores, would negative such view. Perhaps the nearest approach to resemblance would be with the spirillum (or spirochæta) of human relapsing fever, which I mention because it has been alluded to by several observers as regards appearances in the fresh blood of rats and equines, and has in the latter instance led to the organisms being designated as "spirilloids;" Mr. Steel's term seeming the more warrantable, since he found a distinctly recurring pyrexia to attend the presence of the organisms in mules and in animals infected from them. I could myself compare directly the living human parasite with that of the rat, finding no other than an incidental similarity in a peculiar twirling displacement of the red blood-discs, caused by movements of the active organisms present; watching for a time, indeed, both kinds of organisms ceased to move, and becoming rigid drifted away towards the sides of the containing cell, when all resemblance ended; the spirochæta retaining its spiral form, speedily enlarging much and joining with other filaments to form a close network which at its nodes finally blends into a uniform granular, zooglœa-like clump. The contrast here, even so far, with what has been described above of the rat-organisms, is sufficiently marked to indicate an essential difference in their nature: there being manifest, under similar conditions, a

[1] I had purposely omitted in the text any allusion to the so-termed 'hæmatozoa of malaria,' which, after Dr. Laveran and others have been recently recognised by Prof. W. Osler, and by him are in a certain sense associated with the organisms of 'surra' equines and of the rat. This omission was due to the absence of a personal knowledge of the blood-parasites often present in the febrile human subject; and having, since the text was despatched from Bombay, acquired some practical information here, I hope to be allowed to make good the defect in a later Note below.

distinct tendency of the bacterium towards a certain development, and not so of the hæmatozoon. I might add that it is only when the *spirochæta Obermeieri* in its most active state is, as it were, uncurled and variously twisting that it could be mistaken for the *monad;* since when more tranquil it appears as a much slenderer, shorter, and quite uniform spiral thread, darting indifferently backwards or forwards and showing no flagellum at either of its equally obtuse ends. I also note that though morphologically apart, there sometimes obtains (*e.g,* as regards the "surra" organism) a biological resemblance—that, namely, of reproduction through inoculation; but such faculty manifestly pertains to both protophyta and protozoa.

As anthrax in horses is said to be frequent in India and sometimes liable to be confounded with the "surra" disease, it may be worth pointing out that the *bacillus anthracis* has in fresh blood the aspect of delicate homogeneous rods, either straight or slightly curved, with parallel sides and flattened ends; and often joining, as regards mere length as well as breadth they might compare with the "surra" organism, whilst on the other hand their perfect quiescence alone would be distinctive. Inside the body after death, and outside at ordinary temperatures, the rods readily grow and produce their spores.

There now remains a possible identification of the blood-parasite under review with organisms of the sub-kingdom protozoa, forms of which are known to flourish in fluid media of animals with hardly more access of oxygen than obtains in the blood; and it would be with such directly nourished parasitic species, since a differentiation of integument and contents, nucleus or vacuole, mouth or anus, cannot here be made with certainty. Following Leuckart ("The Parasites of Man," translated by Mr. Hoyle, 1886), the identification then might be in advance of the Rhizopods and Sporozoa (Coccidial forms of which may be seen in the rat's liver), and rather with the class of Infusoria, its order Flagellata—the flagellum being always confined to the anterior or oral extremity; next, particularly with the simpler Monad family, including the Genera Ceromonas (with numerous free-living species), and Trichomonas which is wholly parasitic in the higher and lower animals and is characterised by an oval body provided not only with a flagellum, but also with a longitudinal fringe. Thus, without my being in a position to criticise further, the view of Dr. E. M. Crookshank seems reasonable (*l.c.*), namely, that the organism in question may be relegated to this already-recognised genus—its sub-genus *Trichomonas sanguinis,* without resorting to the new terms Herpeto-monas (Kent) or Hæmato-monas (Mitrophanow).

I am aware of certain objections to the view above proposed, and doubtless a decision of this sort is always open to revision; yet in connection therewith as bearing on assigned or possible sources of the parasite-contamination, it seems worth adding (from Leuckart) that these monads commonly live on putrefying substances, and are often found in countless numbers in water, or in living animals, *e.g.,* the large intestine of frogs and toads, the paunch of ruminants and cœcum of pig; also in the genital canals of the snail, the body-cavity of rotifers, the alimentary canal of millepedes and insects, being occasionally so frequent (especially in moths and flies) that whole tracts of the intestine are filled with them.

In the same connection I would here allude to the evident narrow place, limitation, or "endemicity," of both rat-infection and the "surra" disease, with

probably also a distinct "seasonal" variation of prevalence. Strict search on the lines now indicated seems to me promising as regards detection of the source or sources of these infections.

An external origin of the parasite, direct or mediate, being in high degree probable, regarding its mode of entry into the circulation, the successful experiments of Veterinary Officers proving that the "surra" infection is directly communicable by way of the stomach, have an obvious practical bearing; and so, too, the equally prompt success of hypodermic inoculation, which shows the sufficiency of minute wounds of the skin and, presumedly, of the mucous membranes generally, as a favouring local condition. The apparent absence of conveyance of the same morbid state through mere skin-contact, and through air-infection by way of the respiratory tract, should also be considered. It is not known if rats inhabiting the stables of infected horses or mules are primarily or specially affected; but Mr. Steel's negative experiments, quoted above, would tend to show that equines did not derive their disease from the bite of tainted rodents. The hæmatozoon of the rat seems more difficult of communication than the "surra" monad, yet I have shown that it or its germs may be introduced into the circulation, *e.g.*, of the monkey, by way of the peritoneal lymph-sac; my own impression from the data (see above) being that a certain limited development had taken place in the monkey's blood, though this is uncertain since entire blood-discs, while preserving their vital properties, can pass freely along the same lymph-channel (Dr. W. Murray in the Brit. Med. Jour., 29th Jan. 1887), and such are broader and less mobile structures than the full-grown parasite in question. Another conjecture arose from my finding in some rats, at the lower part of the ileum, many active monads apparently identical with species of Cercomonas, and in the duodenum amidst bile-stained mucus, numerous larger ones resembling Trichomonas *sp.*, whose proximity to the common bile-duct suggested the possibility of entry into the liver (where C. has been met with), and thence into the circulation. As it happened there were no organisms in the blood of these rats, further remarks would be merely speculative; and I need only add that the concurrence of extraneous, intestinal, and blood monads, with a conceivable connection of the series, *inter se*, forms a topic analogous to that of such bacterial infection as the "spirillar" of human relapsing fever elsewhere alluded to by me.

Equally cursory in the scarcity of relevant facts must be any comment on the growth and reproduction of this hœmatozoon. The parasitic habit might be expected to modify these processes, as it does mere structure. According to Leuckart the flagellate monads commonly are reproduced by division (longitudinally), or by conjugation, encystation, and the formation of countless spores possessed of much tenacity of life; and in individual cases there has been seen a quiescent state leading to the production of abundant minute swarm-spores.

Here what is known is, first, the co-presence in the blood with abounding grown organisms, sometimes clustering or blending, of numerous small motile particles and frequent cyst-like forms which have the aspect, at least, of reproductive elements at various stages of development, and which nearly all observers have noted (see Dr. Crookshank's figures and some of mine): add, also, that whilst obviously plentiful growth must take place somewhere in the rat and horse's body, hitherto outside the blood no parent-source of the parasite has been detected. Mr. Steel's successful inoculation with fresh blood, in which at the time no parasites were detectable, is quite noteworthy and corresponds with some experience of mine when studying the relapsing fever of man, the explanation being either that grown organisms had (as is possible) been overlooked, or that the new brood had sprung from germs contained in the blood (as also seemed likely in one of my cases). The second point to note is concurrent testimony regarding the advent of the hæmatozoon by intermittent succession of broods. I had not actually seen this in the rat, but apparently it was so in a healthy-looking dog in the possession of Dr. Lewis; and as regards the "surra" infection Dr. Evans had noticed its probability, whilst Mr. Steel has furnished definite affirmative evidence in both natural and imparted disease. Analogous thereto are the events in the human recurrent spirillar infection; only here the malady tends to subside by diminution and delay of the relapses, whilst equine relapsing fever seems always to end fatally. Whatever prove the future explanation of this significant phenomenon, it will apply to both these kinds of blood-infection, and may apply also to the malarial, typhoid, and certain septic species. Lastly, I have to notice the fact that, so far as known, the hæmatozoon is not only limited to the blood-area, but is equally diffused throughout it, and the same datum was clearly ascertained for the spirillum of relapsing fever, by comparative scrutinies of blood taken immediately after death (of infected monkey) from each of the principal regions and viscera of the body.

Before quitting this part of the subject, there might be mentioned certain seeming counterfacts to some of the propositions above surmised. Thus, as regards evanescent quality of the hæmatozoon, this would appear less marked than is stated of monads generally; Lewis in particular having remarked that the rat's organism was not very sensitive to the action of mercuric perchloride, whilst according to Leuckart this salt has in general such a prompt destructive action that its employment therapeutically seems to be indicated. Again, Mr. Steel once failed to propagate the "surra" disease by administration *per oram* of the gastric and intestinal matters of a diseased mule: and, further, what seems adverse to the asserted persistency of monad-germs, the subcutaneous injection of dried tainted blood also failed. Dr. Evans, too, found on two trials no effect after the swallowing of a water-mixture containing infected blood which had been kept for 24 hours and without signs of putridity no longer

showed traces of the parasitic organisms. Analogous hereto was the failure at my hands of dried spirillum-blood to infect, although traces of that bacterium could be seen in the material used; and this circumstance induces me to remark, briefly, that the conditions of infection must really be complex, since three separate factors are necessarily concerned, namely, (1) a certain state of the infecting agent, (2) a certain mode of transmission, and (3) a certain state of recipient or new host.

The final topic I propose to glance at is the character and affinities of the equine disease which is associated with the hæmatozoon under review. The excellent descriptions published of "surra" as prevalent at the E. and W. extremes of India, do not differ more than might be anticipated from innate variability and incidental circumstance. From them it may be gathered that here is a sub-acute, febrile and usually fatal malady; presenting no other special lesion than that of the blood, arising endemically and not spreading by contagion or infection; commonest during and after the rainy season, with no other particular relation to atmospheric states or to the soil: in a given locality the distribution of disease may be irregular, and the outbreaks do not seem to last longer than a few seasons at a time, sporadic cases probably persisting or being introduced afresh. The entire clinical history is not fully known, on account of the non-continuous course of symptoms: at first were noticed fever, with jaundice, petechiæ of visible mucous membranes (eye and vagina), anasarca, sometimes albuminuria, always great prostration of strength and rapid wasting; death by exhaustion alone at the end of a few weeks; and at autopsy so specific coarse lesion seen (Evans). Afterwards it was clearly made out that the pyrexia assumes the form of pronounced (max. t. 105°-7° F.) and well-defined exacerbations of 2—7 days' duration, which are separated by apyretic intervals lasting about as long—the first incubation-period experimentally learnt being 4—7 days; and it is only during these successive "relapses" that the hepatic derangements and blood extravasations become prominent; thrombosis and embolisms may occur: anœmia is very marked (Steel). The duration of the disease, so far as ascertained, varies from about 2 months to perhaps a year; a long latency of the malady seems improbable (Evans) artificially induced disease may end acutely in 21 days or in a month or so (Steel). There are clinical modifications which need not be discussed here; antecedents and sequelæ are not fully made out. The mortality-rate is extremely high, recoveries being apparently unknown. Anatomical lesions seem to be connected with the morbid blood-state, and include localised hyperæmias of the intestinal mucosa, in the stomach sometimes stasis and sphacelus; petechial extravasations; serous and gelatinoid effusions in lymphatic areas, with occasional glandular intumescence: granular and fatty degeneration of solid tissues. The diagnosis of this disease is wholly due to the labour of sagacious experts; by means of the thermometer

the characteristic febrile relapses can be ascertained, and by the microscope the pathognomonic aspect of the blood (see below) ; a period of 10 days' watching may, however, be needful for certainty. In "anthrax" the temperature is persistently high and the blood contains bacilli, and in 9 cases out of 10 this disease is of acute character: whereas in "surra" the malady is more chronic, the pyrexia intermittent, and the blood then charged with "spirilloids" (Steel). Prognosis is unfavourable in the highest degree : and treatment, both general and special, having hitherto proved inefficacious, attention needs be directed to prophylactic and preventive measures.

As to etiology, it is stated that though new to science, "surra" has long been recognised in its haunts, where as a rule enzootic it does sometimes assume an epizootic character (Evans). Adequately sustained enquiry made on the spot, concerning the predisposing and determining causes of the disease is still a desideratum ; only occasional and non-exclusive conditions chiefly of mal-nutrition or mal-sanitation being as yet elicited, there remains to note such more direct agencies as in the popular opinion produce this disease, *e.g.,* the bite of a large brown fly the size of a small bee (classification unknown), the feeding on certain kinds of grass, and the drinking of fouled, stagnant, marshy water as mentioned by Dr. Evans, who himself found flukes in the liver of two "surra" cases examined *post mortem.* The Indian veterinary observers seem unanimously disposed to regard the hæmatozoon as the immediate cause of the disease ; the arguments put forth in support of such view being those held in analogous instances, *e.g.*, the spirillum fever, and also like, but more elaborately conclusive, in the anthrax infection ; it is asserted that this organism is never found in healthy equines (*a*), that it is invariably present in the diseased (*b*), and that its occurrence is peculiar to the diseased (*c*) : further, there is, in the mule at least, the closest connection in time between its usual visible advent and the onset of febrile symptoms, with the additional feature of disappearance of the blood-organism just before the acme and decline of specific pyrexia (Steel) ; and again during the apyretic interval ensuing, it is said that immature growing forms of the parasite are with little difficulty detectable (Mr. Gunn, A.V.D.) ; lastly, the absence of a strict relationship between the apparent number of organisms and the intensity of clinical symptoms would be no valid argument against a real connection here, because such symptoms are really complex phenomena referable to a conjunction of influences and conditions. Judging from all the data, there is, I may add, ample evidence to warrant the assumption that the natural equine disease scrutinised by Dr. Evans and Messrs. Steel and Gunn was one and the same.

The remarkable fact that "surra" is communicable by inoculation and even by imbibition of the tainted blood was determined promptly by its sagacious discoverer, and has been fully confirmed by the next observer, whose more elabo-

rated data are worthy of close attention. Mr. Steel has published three separate accounts, with charts, of inoculated mules and one of the pony, which on comparison with others of the natural mule-disease appear so much alike as, in my opinion, to quite justify his statement that the "surra" blood-infection with all its attendants can be readily, exactly, and exclusively reproduced at will. I am assured that adequate precautions were taken to ensure the absence of a natural acquirement of the disease in these several instances, which therefore, along with Dr. Evans' experiments, will serve to establish a truly fundamental datum.

To myself these observations were of particular interest because of the aggregate clinical and pathological facts embodied in them demonstrating, further, a striking similarity to the phenomena of the spirillum-infection with its attendants as seen in the human subject. This similarity Mr. Steel has the credit of first clearly making evident, whether or not in veterinary medicine his proposed cognomen of "equine relapsing fever" displace the indigenous term "surra": briefly, it consists (as regards inoculated cases) in the defined and but little shorter incubation-period of 4—7 days, with no marked general symptoms and no visible blood-contamination; then follows a succession of pronounced and defined pyretic, and apyretic (incubatory) periods, of which 2 to 6 are shown in the charts, and during which characteristic signs of illness and blood-tainting regularly alternate with abatement of symptoms and disappearance of the hæmatozoon: after death the lesions found, so far as I perceive, being solely those of blood-deterioration without the marks of local inflammation or other organic processes, either primary or in themselves sufficiently marked to account for decease. The differences here, which I note, concern details in the course of the pyretic events; these in 3 of the cases being, if equally pronounced, hardly so defined (*e.g.*, rigors and sweats not named) or so clearly separated by apyrexial days, as is usual in the human disease—the point not however being wholly material, since fatal cases of relapsing fever and other specific infections of man are very apt to be irregular in their course: in the longest surviving of Mr. Steel's mules, where death occurred from an incidental internal embolism, the relapses were better marked, and from this instance is it learnt that the equine infection manifests no such tendency to decline by a deferring and shortening of the febrile attacks, as is manifested in the human infection; thus the mule's series shows a succession of febrile events lasting 7, 3, 4, 3, 4, 6 days, with apyretic intervals of a much shorter and not lengthening succession of 3, 2, 2, 3, 2 days' duration: add to these data the commonly larger number of relapses (17 have been counted), and it will become evident that the relapsing fever of equines is, comparatively, much the more prolonged, intense, and intrinsically exhausting; indeed, so far from possessing a spontaneously abating tendency, once supervening it never departs until life itself ceases, and this, happily, is not the case with the spirillum-fever

of man. Respecting the more prolonged illnesses naturally acquired precise information is as yet but scanty, consisting of three cases given by Dr. Evans in which the animals were destroyed, and of 6 examples detailed by Mr. Steel in which natural demise occurred; of these last 3 had been first entered under a provisional diagnosis of "gastric typhoid" (in allusion to the gastric ulcers sometimes found in Burma cases), dying on the 53rd, 59th, and 93rd day of disease respectively, so far as ascertained; and as the blood-parasites were present in all, there is here, too, evidence of the much longer persistence of the infection than ever was learnt by me as regards the human spirillum-fever. What may be the precise state of the blood and viscera in cases of surra lingering for 6 or 12 months does not appear to be ascertained: nor apparently have recoveries been ever watched throughout, and hence the question of immunity or of an increased liability cannot yet be raised. It is well known that no immunity against re-infection is imparted by an attack of "spirillum-fever."

As to the character of the disease inoculable in animals of different species to the equines and man respectively, available data are but few. I found the spirillum-infection to be communicable only to a quadrumanous animal, in which the blood-parasites with their clinical attendants are even readily reproduced, and, as sometimes happens in the human subject, the first outbreak of illness may prove fatal: it being also remarkable that after the more usual event of recovery, relapses were extremely rare (if ever occurring) in the monkey. As regards the equine disease, Mr. Steel succeeded on first attempt in similarly communicating this to a monkey of probably similar species, whose chart shows the animal to have at first and for 30 days at least truly acquired the specific recurring fever: numerous later relapses are also said to have occurred, but the state of the blood is not recorded, and the monkey died after two months or more with the complication of ulcers and necrosis of the foot, so that possibly (in my opinion) septicœmia had finally supervened, and other data for comparison are desirable. Adverting, next, to disease artificially induced in the dog, a notable divergence of results becomes evident; for whilst like others I failed to communicate the spirillum-infection from man to the healthy common dog, yet three veterinary surgeons have readily transmitted the equine surra-infection to dogs whose blood was, in two instances at least, previously ascertained to be free from the parasites. From these data it appears that a healthy pup infected probably by the mouth on or about 5th October 1880 early showed abundant blood-contamination (Evans), which was again seen at intervals until 3rd November; shortly after this the puppy got the "distemper" and by an injury lost the use of an eye; its blood, however, was free from organisms on 15th January 1881, 12th and 28th February and so late as 24th March (Lewis, second Memoir): the general condition and ultimate fate of the animal being unknown. Mr. Gunn

inoculated a dog on 20th September 1886 and on 24th found many parasites in its blood; the animal after that date, for a period not stated, continued playful and healthy-looking, nose cold, but yet the blood was "crowded more or less with the special organisms more or less numerous as indicated by the internal temperature"—this observer inferring that an "animal may be apparently quite healthy and yet have the disease in his system." Adverting, lastly, to the more precise experiment of Mr. Steel, there is the chart and history of a young dog inoculated and given tainted mule's flesh to eat on 13th January 1881: blood-parasite first seen on the 24th, and on 28th animal dull, had slight cough, and exhibited pain when pressed on the loins—she was very feverish while the blood contained parasites, and the conjunctivæ became pale in the extreme; she was allowed to eat portions of all the mules which died, but her appetite became very capricious—On 25th February the animal was found very dull, feverish and thin, with a swelling on each side of the head—also the left inguinal glands swollen, and the body in general so tender that the animal shrieked when lifted from the ground; in the blood no parasite seen, but many disintegrated corpuscles: the glandular swellings increased, and then soon appeared œdema of the perineum, turgescence of the throat and muzzle, extreme prostration, swelling of the hind quarters, coldness of the body, and death on 4th March (fifty-one days after first inoculation); the chart given showing, first, a series of 6 or 7 distinct febrile exacerbations of 3—5 days' duration and separated by brief apyretic intervals of 2—4 days' duration, but afterwards displaying more irregularities with days of great depression of temperature. Upon enquiry of Mr. Steel, I learn his opinion that here as in the monkey (which died with similar sequelar signs of septic infection) the later illness was really part of the "surra" disease pure and simple; autopsy of the dog was made too late to be of real value.

According to the above comparative data, the "surra" infection like the "spirillar" is readily implanted in the monkey, whilst retaining much of its superior gravity: both instances, however, showing a certain diminution of degree in this transfer. As regards the dog, in which the spirillar contagion is wholly inefficacious, if the first-named results be regarded apart, the case becomes analogous to that of my experimental monkey inoculated with the rat's organism, in so far as showing that the mere presence of the active monads did not induce palpable morbid signs; but the last result indicates, at least, that before deciding on the real effect of the "surra" inoculation precise and prolonged observations should be made. Viewing the whole series together, it seems legitimate to infer that the surra-infection admits of considerably wide limits of intensity, and this independently of the visible quantity of organisms, so far as may be judged from statements of observers: being in some instances (as in equines) almost inevitably fatal, in others (as in the monkey) less immediately so, and, lastly, in the dog not necessarily occasioning early serious illness.

In the course of comparing phenomena, I have noted as a similarity of the equine to the human relapsing fever the occurrence of a ravenous appetite in the sick during even the febrile state, and without much manifest benefit when gratified : also a tendency to intensifying of symptoms (*e.g.*, the jaundice and petechiœ) at the acme of the successive febrile events, according to Mr. Steel, who seems to refer to a clustering of the organisms at this time such as I had connected with incipient sporing of the human blood-spirillum then. The remarkable tendency of the pathogenous flagellated organisms to attach themselves to or attack the red blood-discs, is insisted on, even a disintegration of the corpuscles being thus imagined possible ; such tendency is apparent in the organisms of the rat's blood, without seeming so peculiar, and in less degree it is notable of the fever-spirillum in man. Coarse deterioration of the blood is not apparent in the rat, and not marked in equines nearly so much as in anthrax ; it is little evident in spirillum-fever : minuter changes in " surra " are expressly stated to follow the advent of the parasite, and to consist of increase of white cells, diminution of the red, with an appearance of granular specks in the serum (Evans). If the parasite be viewed as the immediate infecting agent, there becomes evident a similarity of equine to human fever-blood in so far that loss of infecting power ensues on dessication ; but, on the other hand, a contrast with anthrax as regards ingested blood, since in order to infect this must contain spores of the bacillus, whilst germs of the monad will either not suffice or perish far sooner, as Dr. Evans' experiment shows. Surra-organisms (or the spirillar) were not found in the secreta or excreta, though lately quiescent monad-parasites have, it is said, been detected in the urine during apyretic intervals (Gunn); anthrax-bacilli are to be seen in bile, milk, and the urine during illness. Transmission of each of these infections to the fœtus seems to be an uncertain datum : the ready communicability of anthrax and spirillum-infection to man appears in marked contrast to the non-communicability of monad-infection, so far as known (Steel). It is worth adding that the site of successful inoculation of virus in all three cases may present no sign of local irritation ; whilst after unsuccessful attempts local swelling may follow, not necessarily suppurating : such were also noted in unaffected ruminants after surra-inoculation (Steel) ; they were not observed after use of tainted rat's blood.

For some other comparative points of interest reference must be made to the following paragraph and to the appended Table ; my work on " Spirillum-fever " would also afford some aid in following out details.

Amidst the obscurity surrounding a new disease, as yet but imperfectly investigated, it would be warrantable to seek for light by way of analogy with better-known diseases of the same class ; and the search would be more encouraging if there were available a satisfactory standard or type to which to refer. Such a type exists in anthrax, the history of which is full of applications to the case in hand. (See " Leçons sur le charbon par Dr. J. Strauss," in

"*Le Progrès Médical*", 1886-87). Thus, as to general features, anthrax may affect equines on a large scale, preserving there the characters it displays in bovines and sheep, its commoner victims; it also is distributed endemically, being of telluric though not of miasmatic origin; and its severity is hardly less than that of "surra." Next, as to clinical and anatomical diagnosis, "anthrax fever" is the common natural form of the infection, in which the temperature may reach 105°—108° F., being attended with chills and sweats, restlessness, distress, troubles of the circulation, exhaustion, stupor, convulsions and coma; these symptoms extending over 12—24 hours, or sooner ending fatally in young and plethoric subjects: after death the blood is found and remains black, all the internal organs are deeply congested, with ecchymosis in the intestinal canal and stomach and occasional local sphacelus; the lymphatic glands are implicated, and in the horse the superficial and deep connective tissue shows gelatiniform infiltration; the scanty urine and stools may be bloody. The characteristic bacillar aspect of the blood in this disease was but very gradually discriminated. The causation of the malady was for long attributed to collateral conditions—non-sanitary of all kinds, foul emanations, peculiarities of soil (marshy) and combinations of general agencies leading to the view of its spontaneous origin: the special operation of flies (unarmed) on excoriated surfaces was determined by experiment, and it was soon learnt that the disease is communicable by even slight inoculations, though neither contagious nor infectious in the ordinary sense. Rats are with difficulty inoculated, and dogs do not suffer on drinking tainted blood, but the well-known experiments with cultivated bacilli demonstrate that the apparent immunity of some species of animals is in high degree contingent. One attack of anthrax does for a time at least protect against re-infection. The above general view will serve to indicate points of fundamental resemblance between anthrax fever and the relapsing fever of equines and of man; also between these and other infective or septic diseases; there are differences, which, however, seem subordinate or merely of degree and not enough to separate the "surra" disease from other members of the class of acute or sub-acute specific infections. The ultimate difference pertains to the infecting organism in each instance—see appended table,—and as to certain minor variations noted of the hæmatozoon under review in different animals, they are probably no greater than is known of the bacillus anthracis in different species. The indigenous race of horses in India, as of sheep in Algiers, seems far less liable to anthrax than are imported races.

*Concluding remarks on the blood-contamination under review.*—There is to note, first, that Dr. Griffith Evans' discovery in 1880 of the protozoid blood-parasite characterising the equine "surra" malady truly merits the epithet of epoch-making; for until that date no instance was known of the association of acute infective disease, with organised blood-contamination other than the protophytal. And even if this example seem exceptional, it yet possesses much pathological importance as evidence of the close similarity of morbid processes, whether associated with plant or with animal organisms of elementary character; more probably, however, the "surra" affection will not long remain unique, proving to be only the pioneer of a series of the kind. In the abstract there seems no valid reason why hæmatozoa living as hæmatophytes should not be attended with analogous or even identical symptoms of derangement in hosts of the same species; but hitherto such an assertion as this, if doubted, could not be suitably tested, and hence the cognomen of "equine relapsing fever" (employed

by Mr. Steel) must now be interpreted as in a special degree significant. To the best of my recollection, none of the hæmatophytes are harmless tenants of the blood, all being possessed of pathogenetic properties and all, moreover, belonging to the fungus- (or alga-) group of schizomycetes : whereas no such uniformity of property, or of classification, obtains amongst the known hæmatozoa, and hence of these an important differentiation may and has now to be made. Thus, on the one hand, there are the familiar instances of high-grade vertebrates whose blood teems with large and active organisms, without for a time at least any very manifest prejudice to their own health,—these instances, *e.g.*, of birds and many mammals (reptiles and fishes are not excluded) being sometimes quoted in support of the view that all organisms appearing in the blood have but a subordinate meaning: and on the other hand there is the instance of this "surra" disease, in which according to concurrent testimony the horse or mule in whose blood minute organisms appear, of far less formidable aspect than embryo-worms, is doomed to death if less quickly not less surely than an equine infected with the bacillus of anthrax. Limiting attention to these contrasted data, a reasonable explanation of the pathological differences herein noted, might, I think, be found in the circumstance that embryo Trematodes and Nematodes make use of the circulation chiefly, if not wholly, as a means of transport, neither developing nor even growing larger therein—the persistent egg-sac of some being a palpable bar to all but simplest nutritive interchanges; moreover, these filariæ remain but transitorily within the blood-vessels, being probably after a few hours either destroyed or extruded, in neither of which processes is there a conceivable necessity of serious impairment being done to the constantly replenished and purified blood. Some deterioration of the inhabited media is, indeed, inevitable if not demonstrable; and account must be taken of the mechanical lesions occasionally (at least) resulting from impaction of the worm-parent or offspring in the blood and lymph channels of the hosts' body, with consequent œdema and hæmorrhage, either interstitial or free: yet upon consideration it will be evident enough that all such series of morbid changes are of a totally different character to the profounder alterations characterising veritable blood-infections, and could not be deemed reproducible in healthy subjects by inoculation of a blood-drop, as may be the "surra" and anthrax diseases entire.

The last topic I have to allude to is the remarkable contrast of symptoms offered by the rodent and the equine when their blood is equally infested by an organism morphologically identical in the two cases: thus, as already described, the rat then appearing to be altogether unaffected in its health, whilst the horse or mule suffers greatly and is certain presently to die. So far as I am aware, there is not known any strictly similar instance to this amongst the hæmozoic infection, nor any strictly analogous amongst the hæmophytic: yet upon analysis and comparison of all the data, it seems to me that the

peculiarity of the present case rests on the conjunction of phenomena presented rather than upon any single phenomenon, and therefore it may be most convenient to consider separately the pathological state involved and the organism concerned. As regards morbid symptoms, whilst in the horse and mule these become so marked, they are described as being very insiduous at their beginning and liable to apparently complete intermissions. Animals at work have been found to present a highly contaminated state of the blood; others have died suddenly or unexpectedly, and there are some natural variations of symptoms in different provinces and between the horse and mule. These facts are mentioned here with reference to the likely detection, or non-detection, of morbid signs in animals not domesticated and only casually inspected: even in the rat at Bombay, it was not clear to me that infected animals did not at some time and in some manner or degree suffer from their blood-contamination, the probability being that they did so suffer; and as regards the few instances reported of deer, frogs, and fishes in whose blood protozoa have been detected without the remark being made of illness associated therewith, this datum, too, is a negative one and similarly open to uncertainty. I think also that the "surra" data respecting inoculated dogs raise the question whether these animals may not sometimes suffer,—Mr. Steel's experiment pointing to the affirmative, and that with the monkey being likewise noteworthy as a variation from the parent type: and, briefly, there appears to me some indication that, morbid signs amongst the different species of animals being likely to differ, there will eventually be elicited a transitional series of such phenomena concurrent with one and the same monad blood-contamination, beginning with the mildest, *e.g.*, in rat or some dogs and ending with severest, *e.g.*, in equines. Such inference would be supported by analogy with the anthrax infection, concerning which it is said that there is nothing fixed or absolute in the receptivity or the immunity of any one species, and that variations occur amongst even individuals of the same species (Straus. *l.c.* douzième leçon): thus, rats prove to be only exceptionally inoculable, and sheep of Algerian race are naturally almost wholly immune to anthrax, analogous to which fact is the asserted non-liability to "surra" of the ass, whilst the horse and the hybrid mule are both highly susceptible. The present topic might be further illustrated, but it will suffice if a fairly reasonable explanation of some difficulties be already apparent. Perhaps the greatest difficulty, however, yet remains, that, namely, which requires for its solution either the assumption that the hæmatozoon in question has *per se* no essential connection with the symptoms of "surra," being only an attendant or quasi-incidental phenomenon (*a*), or else the admission that the hæmatozoon may retain its morphological aspect and capacity of life whilst parting with all or most of its virulent biological properties (*b*). Regarding, first, the relationship of organism to morbidity, nothing new has yet been elicited

from study of this equine " surra " disease: the original observers were distinctly of opinion that the virus of the disease is represented by the organism, but hitherto the evidence goes no further than was reached by experiment with another infective organism—the Spirillum Obermeieri. Thus in my essays of nearly ten years back I was able to show that the mere presence of the spirillum did not necessarily entail fever or other marked symptoms of derangement; having on five separate occasions of special study detected in the blood this organism for a period of 24—72 hours prior to the onset of the febrile "relapse." This I regarded, and do still, as directly adverse to the doctrine that the spirillum is an epi-phenomenon the result of diseased action; the argument has been summarised elsewhere, and in this place I need only point out the analogy here with the hæmatozoon of " surra," which I understand may sometimes be present early in the horse's blood when no other characteristic symptoms were noted, and which when transmitted to the dog may, sometimes at least, flourish periodically for weeks without the accompaniment of manifest illness; the rat, again, acquires its infection doubtless from a different source and commonly suffers even less. That data of this kind showing a reduced connection—chronological and dynamic—or gradual disconnection between a blood organism and symptoms of disease, are not necessarily indicative of a primary and fundamental separation between the two series of phenomena, has, I think, been fully demonstrated for the longest and deepest studied of all the infective diseases; and to anthrax I must again refer for analogical evidence bearing on the chief seeming anomaly found in the monad-blood-infection under review. In that disease the infective organism is a bacillus which can be isolated and cultivated apart, there under certain conditions becoming enfeebled as to its virulent properties, whilst " morphologically the attenuated bacillus differs but little or not at all from the most virulent bacillus; the filaments, however, seem a little shorter and more divided, and culture-aspects less luxuriant also differ slightly—the spores arising from such culture possess the same modified properties as the mycelium from which they spring" (Straus. XI me Leçon, *l.c.*, January 1887): according, therefore, to this datum, the admission is warrantable that the protozoic infecting-organism while retaining its morphological aspects may yet, under conditions, lose some or most of its virulent properties. It is enough, for my purpose, thus to indicate a possible explanation of the seeming harmlessness of the rat-organsim, as compared with the virulence of the equine, which is yet of indentical aspect. I am indeed aware of the frequent fallacies of analogical inference, but the instance in hand seems unusually free from such risks, if an opinion can be safely based upon the numerous and direct applications of the anthrax-data to the facts of the " surra " disease so far as these are known: the task, however, of demonstrating in detail such applications had best be deferred until there become available more data regarding " surra " of the precise

form of those so laboriously acquired for "anthrax;" and as for encouragement to work in India, it seems to me hard to conceive a more promising field of research than the one which has been the subject of the present remarks.

NOTE.—Respecting the aspect of blood and bacillus of animals inoculated with the attenuated anthrax-virus, I have not met with definite statements: if the organism can be seen to flourish in the blood, whilst producing no other than an occult effect, then the analogy and comparison with the monad-data would be complete: such completeness is not, however, essential to the argument above. It seems that reduction of the virulence of anthrax by transmission through different species of animals is not yet a realised notion; but as by successive inoculations an attenuated bacillus can be raised to full strength, possibly the monad-virus has undergone such augmentation from its lower to highest degrees. Lastly, in employing analogy not against facts so much as in their explanation, no canon of science has been violated; and it is generally admitted that the verified anthrax-data have a bearing far beyond the range of the single subject to which they pertain.

# Observations on Bacteria in Cholera.

BY

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(*With Plates VII and VIII.*)

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The following is a brief statement of the results of microscopical examination of the tissues in nine cases of cholera, which have died at the the General Hospital, Calcutta, during the past twelve months (June 1886 to June 1887), showing, in each case, the duration of illness, the stage the disease had reached, the tissues examined, and the bacteria, if any, found. The patients were all adult European seamen, and the *post-mortem* examination was made in less than an hour after death in every case.

CASE 1.—Duration—23 hours. Stage—collapse. Tissues examined—medulla oblongata kidney and ileum. A few streptococci, generally distributed throughout the kidney amongst the blood corpuscles in the vessels and extravasations (Fig. 8). No bacteria elsewhere.

CASE 2.—Duration—about 48 hours. Stage—collapse, but secretion of urine restored. Two portions of the ileum examined, but they contained no bacteria in their substance.

CASE 3.—Duration—64 hours. Stage—secondary fever, and urine restored. Kidney and ileum examined. No bacteria.

CASE 4.—Duration—22 hours. Stage—collapse with suppression of urine. Tissues examined—ileum, kidney, liver, and spleen. Large curved bacilli were abundant both within and without the crypts of Licberkuhn and in the adenoid tissue surrounding them. A few also below the muscularis mucosæ (Fig. 1). No bacteria in the other tissues.

CASE 5.—Duration—69 hours. Stage—collapse and urine suppressed. Tissues examined—kidney, lung, ileum, and colon. There were abundant small curved bacilli, more or less mixed with straight bacilli and micrococci, in the crypts of the ileum, but none outside the crypts or in the adenoid tissue (Figs. 2, 3, and 4). A few micrococci in the blood vessels of the submucous tissue. Large, very slender, filiform bacilli in a congested patch in the colon (Fig. 6). Streptococci in the vessels and amongst pus corpuscles in the air cells of the lung (Fig. 7). No bacteria in the kidney.

CASE 6.—Duration—150 hours. Stage—secondary fever, but urine not restored. Tissues examined—stomach, supra-renal capsule, kidney, spleen, and

medulla oblongata. In a congested patch in the stomach there were abundant short straight bacilli, many of which presented deeply stained spots (spores ?) in their interior. No bacteria in the other tissues.

CASE 7.—Duration—28 hours. Stage—collapse. Tissues examined—kidney and ileum. No bacteria.

CASE 8.—Duration—206 hours. Stage—secondary fever with delirium, urine restored. Ulcers of the stomach only examined. Abundant straight bacilli, presenting deeply stained spots, or spores, in their interior, on the surface, and in the depth, of the ulcers and the mucous membrane surrounding them (Figs. 9 and 10).

CASE 9.—Duration—155 hours. Stage—secondary fever, urine restored. Tissues examined—vermiform appendix and purulent focus in the lung. Numerous spirilla in the crypts at the root of the appendix (Fig. 5), and micrococci in the lung.

From this statement it will be seen that some portion of the intestines was examined in seven cases, and that in four of these no bacteria were found in the substance of the mucous membrane. In the three other cases bacteria were present in the depth of the mucous membrane, but they differed remarkably in character or distribution in each case (Fig. 1). In No. 4 of the table the bacilli were of large size, distinctly curved, and abundant, not only in the interior of the crypts of Licberkuhn, but also on their exterior surface, and in the adenoid tissue surrounding them. They had also penetrated in many places below the muscularis mucosa into the sub-mucous tissue. In No. 5 the bacilli were smaller and more curved, and in portions of the ileum from the neighbourhood of the ileo-cacal valve they were very abundant throughout the whole length of the cavity of the crypts, but there were none on the exterior of the crypts or in the adenoid tissue (Fig. 2). In the spaces between the villi and on their free surfaces, as also, in most cases, just within the mouth of the crypt, the curved bacilli were more or less mixed with micrococci and minute straight forms, but in the deeper parts of the cavity they were almost pure (Figs. 3 and 4). In the vessels of the sub-mucous tissue there were a few minute micrococci. In the colon of the same case there were several slightly raised, smooth, deeply congested patches, in which the microscope showed the crypts had been completely destroyed and the whole depth of the mucous membrane infiltrated with leucocytes, blood-corpuscles, and peculiar filiform bacilli which were very numerous and penetrated down to the muscularis mucosæ, but not below it. These were very long and narrow, tapering to a fine point at either end, and stained faintly and unevenly along their length, so that they presented an irregular beaded appearance (Fig. 6). They have some resemblance to forms described by writers as involuted cholera bacilli In No. 9 only the vermiform appendix was examined, but in the crypts at its attached end there were numerous spirilla as well as detached curved bacilli (Fig. 5).

In cases of secondary fever it is not uncommon to find the lower lobes of the lungs œdematous and presenting here and there small purulent foci. In No. 5 this condition was very marked, and sections through one of the abscesses, about the size of a walnut, showed numerous micrococci, generally in the form of a streptococcus of three or four heads, both within the vessels and amongst the pus corpuscles which filled the air cells (Fig. 7). The lungs in No. 9 were in a similar condition.

In No. 1 there were micrococci in the blood in the vessels, and in extravasations amongst the tubes, of the kidney (Fig. 8). In five other cases a most careful search failed to show any bacteria in the kidney.

In two cases, both of long duration and with marked secondary fever, there were ulcers in the stomach. In No. 6 there were two slightly raised deeply congested patches with smooth bare surfaces, in which, under the microscope, the glandular tissue appeared to be completely destroyed and infiltrated with leucocytes, blood-corpuscles, and numerous straight bacilli, some of which had a spotted appearance, as in the next case. In this, No. 8, the ulcers were three in number, of the ordinary form, with raised undermined edges and a necrosed slough in the centre. The surface of the ulcer and of the mucous membrane near it was thickly covered with clusters of straight bacilli, which penetrated deeply into the mucous membrane and took the place of the epithelium (Fig. 9). These bacilli were stained deeply at either end, and in the case of the longer ones at one or more points in the length, so that they had a spotted appearance as if they contained spores (Fig. 10).

The following *methods* were employed:—The tissues in all cases were removed as soon as possible from the body and placed at once in alcohol, and, after hardening in this, they were thoroughly infiltrated in paraffin and cut up with a Cambridge rocking microtome, to enable the sections to be fixed upon a cover-glass with a solution of celloidin in clove-oil (Schällibaum's section-fixing process, *vide* Lee's Vade Mecum, section 275). The cover-glass is painted with the thinnest possible film of this solution, and, after the sections in paraffin have been laid out upon it, warmed gently over a spirit lamp or on a water-bath, so as to melt the paraffin and evaporate the clove-oil. By this means the sections are firmly fixed upon the glass and it can be afterwards put through the staining processes in the same manner as an ordinary cover-glass preparation, the paraffin being removed by naphtha or other solvent. Any stain that the celloidin takes up is quickly removed by alcohol. For clearing, clove-oil must generally be avoided because it loosens the sections, and, as a rule, oil of origanum has been used, which has also the advantage of not dissolving out aniline stains. By this fixing process sections so friable as those of a lung infiltrated with pus can be easily stained and mounted without disturbance of the delicate tissues or loss of pus corpuscles, &c., while sections of the intestines can be laid out flat and

untwisted with all the sloughs, mucous, blood and other corpuscles accurately *in situ,* and, consequently, can be more satisfactorily examined for bacteria than when freezing or other methods of making sections are adopted. Three, four, or even more sections, according to their size, and either consecutive or otherwise, can be fixed upon a large cover-glass and thoroughly searched and compared. For staining the intestines alkaline methyl-blue (Schutz or Löffler's solution) or watery solution of Spiller's purple, has been used, while in the case of the other tissues either these or some stain by the Gram method.

CALCUTTA,
*July 1887.*

# On the Phenomena of Propagation of Movement in Mimosa pudica.

BY

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In one of the most recent English works on Vegetable Physiology[1] the author expresses himself as follows in regard to the question of the propagation of movements in *Mimosa pudica* and similar plants :—

"It appears from these considerations that the theory which regards the transmission of a stimulus as a mere propagation of a disturbance of hydrostatic equilibrium is not satisfactorily established. The theory is altogether too purely mechanical to account for so remarkable a phenomenon in a living organism, and we must endeavour to establish for it some other explanation which, while borne out at least as fully by the facts, will be more in harmony with our conceptions of the organisation of living beings."

An attempt is next made to show that such an explanation is to be found in the continuity of the protoplasm throughout masses of vegetable tissue forming tracks for the propagation of irritation from one organ to another.

The object of the following pages is to show that this theory is by no means as fully borne out by the facts as that for which it is substituted, and that there is a great mass of phenomena which, however they may conflict with current conceptions regarding organisation, clearly indicate that fluctuations in fluid tension, acting along with structural peculiarities of different masses of tissue, are the principal, if not the sole, cause to which propagation of movement must be ascribed. Details of such phenomena will be found in the tables showing the results of a very extensive series of experiments on the movements in *Mimosa pudica,* and they appear to me to justify the following theses :—

I. *The degree to which propagation of movement occurs runs parallel with the degree to which facilities for the extension of fluctuations in the fluid tension of the tissues are present.*

II. *The direction in which propagation of movement occurs is in many cases that in which the occurrence of fluctuations in the fluid tension of the tissues can be shown to occur, whilst it cannot be accounted for as the result of protoplasmic conduction.*

III. *The order of events in instances of propagation of movement is in*

[1] Lectures on the Physiology of Plants. By Sydney Howard Vines, M.A., D.Sc., F.R.S., Cambridge, at the University Press, 1886.

*many cases inexplicable on any theory of continuous propagation of protoplasmic irritation, while it is readily explicable as the result of fluctuations in fluid tension acting upon masses of tissue of different structural characters and holding different relations to surrounding parts.*

Proceeding to the consideration of each of these in order, we have first to examine into the evidence in favour of the first. In doing so data might be derived from almost every table, but in the first place special attention is invited to those in Tables I to VII, showing the results following section and heating of distal pinnules, to those in Table VIII, showing the results of simple mechanical stimulation, and to those in Table IX, showing the phenomena attending mechanical obstruction to depression of the petiole on action of the primary pulvinus.

Table I is a general table of the results of experiments carried out at different times of year and under various conditions of temperature and atmospheric and telluric moisture. It includes details in regard to 100 cases of section of the tips of distal pinnules. The movements resulting from the injury were in 94 cases limited to the injured leaf, and in only 6 did they extend to one or more other leaves on the axis. In regard to 84 cases it is recorded whether the leaf was exposed to direct sunshine or was in shade at the time of section. In the 45 cases in which the leaf was exposed to the sun, action extended over the entire leaf in 41 instances, and in some extended to one or more other leaves. In the 4 cases in which the extension was not complete the tip of one pinna failed to act in one, an entire pinna in two, and one pinna and the primary pulvinus in one. In the 39 cases in which the leaf was in shade at the time of section action failed to extend over the entire leaf in 38 cases, being in 31 limited to the injured pinna alone or to it and the primary pulvinus, in 6 cases extending incompletely to other pinnæ and in one case only extending completely throughout the entire leaf.

Table II shows the results of section of distal pinnules of leaves in shade shortly after a fall of rain. It includes details regarding 20 experiments. In no single case was there any extension of movement beyond the injured leaf; in 10 cases action was limited to the injured pinna or to it and the primary pulvinus, and in the remaining cases it was never complete throughout the entire leaf.

Table III shows the results following section of distal pinnules of leaves in shade after a continuous period of heavy rainfall. It includes data of 10 experiments. Here, again, in not a single instance was there any extension of action beyond the injured leaf. In 6 cases action was limited to the injured pinna or to it and the primary pulvinus, and in all of them the action in the cut pinna was incomplete. In none of the 4 cases in which action extended to other pinnæ than the injured one was it complete or even well developed in them.

Table IV contains the results of 29 experiments on section of pinnules in different parts of pinnæ in leaves in shade. All of them were conducted on one morning, after a night in the early part of which there had been a heavy fall of rain. In none of them was there any extension of action beyond the injured leaf, and in none was the action in the latter complete.

Table V shows the results in 15 experiments on dry[1] leaves fully exposed to direct sunshine. Here, in 13 cases, the action was complete throughout the injured leaf, and in one of them it extended to another leaf. In the two remaining cases it extended to all parts of the leaf, but was not complete.

Table VI contains the details of results in 40 experiments, conducted under various external conditions, in which heat was applied to the extremities of distal pinnules. In only 6 of them was there any extension of action beyond the leaf primarily affected. In 34 cases it extended over the whole of the primarily affected leaf. In the remaining 6 cases in which the action was limited to the primarily affected leaf, action failed in the primary pulvinus in 2; in the others action occurred in the primary pulvinus but failed to extend completely over the remaining pinnæ.

Table VII shows the results obtained in a series of 10 experiments on the action of heat on the distal pinnules of leaves of dry plants.[1] Here, in 7 instances, action was extended to one or more leaves on the axis besides the one originally affected, and in the remaining 3 cases the action in the leaf was complete throughout in two and almost complete in the third.

In Table VIII the results of 3 distinct sets of experiments on the effects of influences producing movement apart from any abnormal discharge of fluid from the tissues are detailed. It relates to 30 experiments. In 10 of these full depression of the petiole was induced by pressure on its upper surface, in 10 a similar effect was induced by impact on the undersurface of the primary pulvinus, and in the remaining 10 action in individual pinnules was induced by elevation or depression of their tips. In the first set of cases full depression of the petiole was unaccompanied by any further results in 9 cases; in one case slight action in the secondary pulvini occurred as a secondary phenomenon. In the second series slight action in the secondary pulvini occurred in two cases. In the third series the action was throughout purely local.

In Table IX we have the results obtained in 24 experiments, conducted on one morning, on the effects of section of the tips of distal pinnules of leaves, in which in 12 instances depression of the petiole was mechanically obstructed, while in 12 it was fully carried out. In the 12 cases in which depression was mechanically obstructed, the action in 9 cases was limited to the injured pinna and the primary pulvinus, whilst in those cases where it extended further it was

[1] The term "dry" is here used to indicate that the leaves were free from any visible adherent dew or rain.

extremely limited in its distribution. In the 12 cases where depression of the petiole was fully and freely carried out, action extended in various degrees to other pinnæ than that primarily affected in no less than 10 cases.

In regard to the two latter tables, it may possibly be affirmed that the limited number of the experiments detailed, and the fact that in each case they were conducted on a single occasion, detracts from the value of the data. To any such objection all that I can reply is, that the only reason why the details of a larger number of experiments conducted at different times have not been given is that it appeared to be undesirable to increase the bulk of the tables, already sufficiently cumbrous, by a mass of monotonous repetitions of identical results. It may, however, be as well to give a little further information in regard to the points specially dealt with in these tables. With regard to the effect of movements induced by the purely mechanical agency of pressure or impact apart from anything causing abnormal discharge of fluid from, or abnormal obstruction to, the access of fluid to the tissues, all that I can say is that, after having tried a very large number of experiments on the subject, I am fully convinced that they are invariably almost, if not absolutely, localised where the experiments are conducted under conditions such as those prevailing in Calcutta. By means of upward or downward pressure on the petiole or upward or downward pressure on the primary pulvinus, extreme petiolar depression may be certainly induced with an entire or almost entire absence of any further result.[1] By judiciously applied impulse, either all the pinnules of all the pinnæ alone, or all the pinnules of all the pinnæ and all the secondary pulvini may be caused to act without the faintest trace of action in the primary pulvinus. Finally, by applying upward or downward pressure to individual pinnules in a pinna, absolutely localised movement in them alone may be secured, and to such an extent is this the case that numerous isolated pinnules on both sides of one and the same pinna may be caused to act without any propagation of movement either centripetally, centrifugally, or transversely.

With regard to the phenomenon of minor extension of action in leaves where there is a prevention or diminution of the petiolar depression consequent on action in the primary pulvinus, it may be stated that the experiments tabulated are merely given as showing the results constantly observed in other cases in which obstruction either occurred naturally due to the relation of the leaves and axes in dense masses of the plant, or was artificially induced by means of ligatures confining the petiole more or less completely to its erect position.

So much for the general results of the tabulated experiments, but we have now to consider their bearing on the question of the cause of the propagation of movement from one part to another of the plant. At the very outset there is

[1] The phenomenon of further action where it does occur may be best considered in connection with the events recorded in Table IX.

one thing which cannot fail to strike any one who is in any degree acquainted with the subject, namely, that in the experiments in regard to which details are here given, the extension of the action under the influence of so-called stimulant agencies, such as section and heat, is curiously limited as compared with what it would appear to be in Europe according to the ordinarily accepted authorities. Now, there can be absolutely no doubt that this limited extension is the normal phenomenon in plants grown in the open ground in Calcutta under similar conditions to those to which the plants of the experiments were exposed. But if this be so, the difference in degree of propagation of movement in Calcutta and Europe must be due either to differences in the degree of irritability, conductivity or contractility of the protoplasm of the tissues, or to the presence of differences in degree of conditions favouring the occurrence of fluctuations in degree of fluid tension throughout the tissues. According to the former view it must be assumed that in the plants in Calcutta growing in the open ground and under conditions so favourable as to lead them to run wild and assume the character of troublesome weeds, the protoplasm is less irritable, or less conductive or less contractile than it is in pot-plants in European laboratories and conservatories. The necessities of the theory remain, however, the sole ground for any such assumption. On the other hand, on the view that the propagation of movements is essentially due to extension of fluctuations in tension of fluid in the tissues, an explanation of the phenomenon is ready at hand. In plants growing as they do in Calcutta, the facilities for rapid and extensive root absorption must be much greater, and the tendency to loss by evaporation much less than in plants with roots confined within limited areas of soil in pots and exposed to the relatively dry air of laboratories or conservatories.[1] Any interference giving rise to abnormal escape of fluid from the tissues either as the result of ruptured continuity as in cases of section, or of diminished capacity to retain fluid, such as is apparently present (*vide* page 95) in cases of heating, must almost inevitably give rise to movements, the extension of which over larger or smaller areas of the tissue will be proportionate to the facilities for rapid removal of fluid from the site of escape and for a renewed supply to make good the loss. In plants under conditions such as those prevailing in Calcutta we should expect, then, to find, what we actually do find, that extensive propagation of movement is a rare phenomenon as compared with what it is in European plants. It appears thus that in this instance the theory of propagation of movement as essentially dependent on protoplasmic continuity fails to furnish an explanation of a phenomenon readily explicable by the other view, and that this phenomenon is one indicating that the extension of movement is proportionate to extension of fluctuations in fluid contents of the tissues.

[1] In Calcutta, as in the lower parts of the Gangetic delta generally, we have to deal with an area in which the soil is at all times saturated to within a few feet of the surface, the atmospheric humidity constantly high and frequently excessive, and the rainfall great.

Taking the data in the separate tables, we find additional evidence of this in the following phenomena. In Table I there is evidence to show that the extension of movement is related to exposure to sunshine or shade, the tendency to extension being, under otherwise like circumstances, much greater under the former than the latter condition. But exposure to direct sunshine is certainly exposure to increased evaporative loss of fluid. This, of course, needs no demonstration, but if it did, an examination of the condition of the leaves of any mass of the plant, before and after it has been exposed to direct sunshine, ought to be sufficient to meet all requirements. In a vigorous mass of the plant growing under favourable circumstances, we find that it is in the early morning, and whilst the leaves are still moist with dew, that the condition of expansion is at a maximum. As the sunshine strikes on it, and as the leaves gradually lose their adherent moisture, we find that the expansion tends to diminish, and, under continued exposure, in many cases becomes very conspicuously less than it was. Not only do we find slow, insensibly progressive movements taking place under the influence of the sunshine, but, under certain circumstances, rapid, large movements occur similar to those resulting from so-called stimulation of the tissues. If a mass of the plant be carefully watched just as it is struck by the sunshine, such movements can frequently be observed to take place in large numbers. Especially conspicuous are those taking place in the case of young, terminal and only partially-expanded leaves situated at the tips of axes. These are, as a rule, so long as the plants remain in the shade, in a condition of extreme erection, but after a brief exposure to the sunshine in many instances, quite apart from any other appreciable exciting cause, become suddenly depressed, due to action of their primary pulvini and depression of the petioles. Similar movements may also be seen to occur, under like circumstances, in other portions of the leaves. They appear often very conspicuously in the case of leaves in a condition of partial recovery from the effects of application of heat to distal pinnules. Recovery in such cases usually commences with elevation of the petiole, followed by divergence of the pinnæ, and gradual centrifugal expansion of the pinnules. In leaves in which recovery has advanced so far in the shade that the basal pinnules have begun to diverge, the access of direct sunshine is often followed almost immediately by movements of relapse in varying degree, in some cases confined to the pinnules or secondary pulvini, but in other cases complete and involving the primary pulvinus and leading to renewed petiolar depression. Similar phenomena present themselves in the case of the leaves on detached branches kept in a saturated atmosphere beneath bell glasses, if the latter be carefully removed and direct access of sunshine permitted.

Exposure to direct sunshine implies increased facility for evaporative loss, and we find from the experiments that it also implies increased tendency to extension of movement. The theory that extension of movement is

dependent on protoplasmic continuity must assume that the sun's rays elevate the irritability, conductility, or contractility of the protoplasm without affording any evidence that they actually do so, the other theory can point to direct evidence of increased facility for evaporative loss and therefore increased facility for extension of fluctuations in fluid content of the tissues running parallel with increased tendency to extension of movement.

The table not only contains evidence of the association of increased tendency to extension of movement with exposure to conditions favouring loss of fluid from the tissues, but further a certain amount of direct evidence of the association of special limitation of extension with the presence of conditions favouring supply of fluid to, and obstructive to loss of fluid from, the tissues. In experiments 54 to 57 inclusive we have a set of cases in which the phenomena of movement in leaves in the shade and exposed to rain at the time of experiment are detailed. In every one of these cases we find that the phenomena of movement were extremely limited in their extension. In none of them did they extend beyond the injured pinna and the primary pulvinus, in two cases movement was confined to a limited number of pinnules, and in two more to a limited number of pinnules and to the primary pulvinus.

The next two tables (II and III) contain evidence of a like nature. They show the phenomena presented by leaves in the shade and after recent rainfall on section of distal pinnules. In both we have clear evidence of limitation of extension of movement, and there are even indications of a direct relation between the degree of limitation and the amount of moisture to which the plants were exposed. In the case of the experiments in Table II the plants had been exposed on the previous evening to heavy showers, while those in Table III were carried out after an evening and night of continued rain, involving, at all events, a greater supply of soil moisture, and in association with this we find indications of the presence of greater limitation in them than in the others. In Table II we have 20 experiments with limitation of action to the injured pinna or to it and the primary pulvinus in 10; in Table III we have 10 experiments with such limitation present in 6 cases.

According to the theory of the protoplasmic origin of extension of movement, we find now that it must be assumed, not only that sunshine raises the activity of the protoplasm in this respect, but that the addition of moisture lowers it. According to the other theory the phenomena differ from one another in the different instances, because of the presence of demonstrable differences in the facilities for loss and supply of fluid.

Table IV contains evidence of a similar kind to that afforded by the two previous tables. The series of experiments, the results of which are embodied in it, was conducted on a morning following a night in the early part of which there had been a heavy fall of rain. The morning was cloudless, but slightly hazy and the direct sunshine had not yet reached the plants. The leaves were

in a state of full expansion and had been thoroughly washed by the rain, but the amount of moisture adhering to their surfaces was trifling as compared with that present after any normal night of heavy dewfall. The extension of movement was in almost every instance very limited, in no case was it complete throughout the injured leaf, and in no case was there any extension beyond it.

The data afforded by Table V are to be regarded as supplementary to those in Table I, complementary to those in Tables II, III, and IV. We have here to deal with the phenomena presenting themselves in 15 cases of section of leaves from which the sunshine had already completely removed the adherent dew, but which were still in a condition of full expansion. The results contrast strikingly with those in the three previous tables. The extension of movement was much more considerable, complete movement occurring throughout the entire leaf in 13 cases, movement extending to all parts of the leaf in the remaining two, and an extension to another leaf on the axis occurring in one instance. We have here, again, evidence of the association of increased extension of movement with increased facilities for the occurrence of extensive fluctuations in the fluid contents of the tissues.

The data contained in Table VI show that the phenomena following the application of heat to distal pinnules only differ in degree from those following section. They show a distinct tendency to greater extension of movement, but that, under conditions such as those prevailing at the time of experiment, the extension was in any case comparatively limited, and in the great majority of instances was confined to the injured leaf. The 40 experiments, the results of which are embodied in the table, were, in the great majority of instances, conducted in the morning, and when the plants were still loaded with dew and not as yet exposed to direct sunshine, and, under these circumstances, we find that in only 6 cases was there any extension of movement beyond the leaf to which heat was applied. The results here contrast strikingly with those in the following table.

In this, Table VII of the series, we have to deal with 10 experiments. These were all conducted on a cloudy morning between 8 and 9 A.M., but before there had been any direct sunshine. The plants were in a state of full expansion, but the leaves were quite dry, there having been a minimum of dew deposited during the course of the previous cloudy night and no rain having fallen for 10 days previous. Under these circumstances, we find that the tendency to extension of action was manifestly increased, as in 7 instances action extended beyond the leaf primarily affected to one or more others along the course of the axis, and in every case the action, at all events, extended over the whole of the primarily affected leaf. Here the influence of direct sunshine as influencing protoplasmic activity is directly excluded, and on the protoplasmic theory we must assume that relative desiccation gives rise to augmentation of the protoplasmic activity of the tissues. As in the previous cases

however, there is no proof in support of this but the necessities of the theory; whereas the phenomenon is capable of serving as direct evidence in support of the other theory.

It may possibly be objected here that in cases of application of heat to pinnules there is no evident reason for the occurrence of abnormal loss of fluid to an extent corresponding to the ensuing phenomena of action, and no such manifest occurrence of escape of fluid as that taking place in cases of section in which the exudation of drops of fluid from the cut surfaces can almost always be readily recognised. The evidence that an abnormal escape of fluid does occur under the influence of heating of the tissues is, however, clearly afforded by Table X, in dealing with the details of which a further reference to this point is made.

The data contained in the next table, No. VIII, of the series are of a nature which it will be hard for the protoplasmic theory to account for. In them we find that where no abnormal escape of fluid from the tissues is connected with the so-called stimulation, there is an almost absolute localisation of movement to the area directly affected. The only exceptions to the localisation which I have observed are in certain cases where action of the primary pulvinus is primarily induced. In such cases a certain degree of subsequent action in the secondary pulvini or in the pinnules may occasionally be observed to occur. The occurrence of extension of movement in these cases, as the data in the next table will show, is, however, no evidence of the occurrence of any protoplasmic extension of activity, but is, on the contrary, to be accounted for as the result of obstruction to the normal fluid supply of distal parts connected with extreme flexion of conducting tissues. Advocates of the protoplasmic theory may possibly affirm that the extreme localisation of movement in cases of purely mechanically induced action is the result of a minor degree of stimulation of the tissues in such cases, pure mechanical impulses being less irritant than ruptures of continuity or thermal agencies. But are we to suppose that in cases where every pinnule and all the secondary pulvini have acted under the direct influence of pure mechanical impulse there has been less stimulation than when the tips of one or two distal pinnules alone have been divided or heated? According to the protoplasmic theory we must do so, as no extension of action occurs in one case and extension is the normal phenomenon in the other. Here, as in all the previous cases, the protoplasmic theory requires to be supported by the assumption of a hypothetical condition; whereas the other theory can point to the presence of an actual one to account for the phenomenon under consideration.

The data, embodied in Table IX, have been already alluded to in dealing with those of the previous one. They are those obtained from a series of experiments which were all conducted on one morning, but the results agree with those obtained on many other occasions so closely in regard to the

main phenomena that they may, I believe, be regarded as normal. They very distinctly indicate the existence of a direct relation between the degree of petiolar depression and the extension of movement. In cases where petiolar depression was fully carried out, we find that extension of movement was greater than in cases where petiolar depression was suppressed or obstructed. Now, it is very hard to see why the degree of petiolar depression should affect the protoplasmic activity of the tissue, so as to interfere directly with the extension of stimulation of the protoplasm. It is not as though the failure in petiolar depression in these cases had been due to failure in action in the primary pulvinus, for in all these cases the primary pulvinus acted and did its utmost to secure depression. But on the theory that extension of movement is a matter of extension of fluctuations in the fluid content of the tissues, we can readily explain the phenomenon, for in cases where full depression of the petiole is free to occur there is necessarily extreme flexion of the tissues of the primary pulvinus, and such flexion must imply obstruction to the onward flow of fluid from the axis in direct proportion to the degree which it attains. But in cases in which the abnormal facilities for escape of fluid induced by experimental interference are approximately alike, as they presumably were in the set of experiments referred to in the table, the resultant phenomena ought to be in proportion to the facilities for the supply of fresh fluid to the tissues. In those cases in which there is obstruction to such renewal of fluid, the extension of movement ought to be greater than in those where obstruction is absent, or is present in minor degree, and these are precisely the phenomena which we find actually presenting themselves to observation. The phenomenon of a certain degree of action in the secondary pulvini in some cases where action of the primary pulvinus and petiolar depression have been caused by means of purely mechanical agencies which are normally followed by purely localised action, finds its explanation here.

Taking the data in the tables which have now been considered as they stand, I believe them to show that while the protoplasmic theory requires a continuous series of assumptions to bring it into accordance with facts, the other theory derives direct support from them, and that the thesis of the parallelism of extension of movement with facility for the extension of fluctuation in the tension of fluid in the tissues is amply supported.

The evidence in favour of the second thesis, that the direction in which propagation of movement occurs is in many cases that in which the occurrence of fluctuation in the fluid tension of the tissues can be shown to occur, whilst it cannot be accounted for as the result of protoplasmic conduction, has next to be considered. It is principally contained in Tables X and XI, showing the phenomena occurring in cases of the application of heat to the course of axes and those following section of the tips of axes.

Table X contains details regarding the effects following the application of

heat to axes, the application in some cases being carried out by means of flame, in others by contact of heated metallic surfaces, and, in still others, by means of focussing the sun's rays on the surface with the aid of a lens. The general results were not in any case alike. There was always a more or less extensive propagation of movement along the course of the axis, but while in all cases centrifugal propagation manifested itself and, as a rule, was extensive, in only 18 cases was there any centripetal action, and in these of very limited extension only.

Table XI deals with the effects of section of the tips of axes in 38 cases in which the phenomenon of propagation of action manifested itself in various degrees of intensity, the extension being sometimes very limited, at others very widely diffused. In dealing with this table it must be pointed out that phenomena of propagation of movement are by no means necessarily present in every case of such distal sections. They do not by any means occur with the same constancy with which those following heating of axes occur. The table, therefore, is to be taken simply as illustrative of effects frequently following distal section, and not as illustrating an invariable phenomenon.

In proceeding to consider the bearing of the data in the first of these tables on the question of the causation of propagation of movement, the most striking phenomenon presenting itself to observation is that of the rarity and limitation of centripetal as compared with centrifugal propagation. In only 18 cases was there any centripetal propagation, and in 15 of these the action was limited to a single leaf, while in the remaining 3 cases it did not extend beyond two leaves. In the same cases centrifugal propagation manifested itself along the entire course of the axis beyond the point of application in 13 cases, and in the remaining 5 the amount of centrifugal extension exceeded that of centripetal action. In the remaining 50 cases in which centrifugal propagation alone manifested itself, movements took place in all the leaves along the axis beyond the site of application in 30 cases, and in the remaining 20 cases the propagation was, as a rule, relatively extensive, being only in 3 instances limited to a single leaf, and in the others manifesting itself in from 2 to as many as 14 leaves.

Now, how far are these phenomena explicable by the theory of propagation as the result of conduction of impulses along continuous masses of protoplasm? If they are to be explained by it, it is necessary to assume that the protoplasm has a greater conductive power centrifugally than centripetally, for it can only be as the result of this that propagation occurs so much more frequently and extensively in the centrifugal than in the centripetal direction. This difference in conductivity must, moreover, be very great to account for the great general differences in the degree of extension and for the frequency with which centrifugal action alone manifests itself, even where the site of application is very close to an inferior leaf and relatively remote from any superior one. But there is, so far as I am aware, absolutely no independent evidence in favour of any

such assumption, whilst there are many facts to show that it is quite untenable.

In the first place we have the phenomena presenting themselves in cases, such as those which are embodied in the earlier tables, of the occurrence of ready and certain centripetal propagation of movement under the influence of agencies acting on the terminal portions of leaves. These tables, moreover, not only demonstrate the facility with which centripetal action occurs, they also show that, under certain circumstances, it takes place more readily and fully than centrifugal action. On consulting the statement of results in experiments 21 to 29 of Table IV dealing with the effects following section of the tips of the central pinnules of pinnæ, it appears that centripetal action, as a rule, both preceded and was more complete than centrifugal action. The number of the cases cited is, no doubt, very limited, but the results may certainly be taken as typical of those occurring under like circumstances, and distinctly prove that there is no general tendency to centrifugal rather than centripetal action. If it be now objected that these experiments refer to leaves and not to axes, and that therefore they cannot be fairly cited as evidence in regard to phenomena manifesting themselves in the latter, the data in the table which has next to be dealt with afford a sufficient answer.

This table, No. XI of the series, contains details regarding 38 cases in which the tips of axes were removed by careful section, and clearly shows that very extensive centripetal action readily occurs under certain circumstances. In order, then, to explain the phenomena occurring in cases of the application of heat to axes, the protoplasmic theory must assume, not merely the presence of greater centrifugal protoplasmic conductivity, but also that the directional conductivity of the protoplasm is modified according to the nature of the influence which gives rise to movement, section of pinnules, or of the tips of axes giving rise to a greater amount of centripetal conductivity than the application of heat to axes is capable of doing. Here, again, the facts do not support the theory, but, on the contrary, necessitate the introduction of unfounded assumptions to bring them into accordance with it.

It now remains to determine how far the other theory accords better with the phenomena of excess of centrifugal propagation in cases of heating of axes, and at the same time of extensive centripetal propagation in cases of terminal section. There can, I believe, be little doubt that it does so. In dealing with the axes, we are dealing with a series of conducting channels along which fluid is constantly passing, the current being kept up due to constant losses of large amounts of fluid from the leaves and other distal parts and constant additions of fresh fluid from the roots. If now any abnormal agency give rise either to obstruction of such channels or to abnormal discharge of fluid from them at some point in their course, fluctuations in the amount of fluid and, therefore, fluctuations in the tension in the system of channels must be induced.

So far as abnormal escape of fluid is concerned it must tend to fluctuations of depression throughout the entire system, but specially beyond the point of escape. So far as obstruction is concerned, on the other hand, it must tend to the establishment of negative fluctuations over all the area beyond the site at which it occurs and of positive fluctuations over all centrally situated areas. Now, in cases of application of heat there is reason to believe that both obstruction and abnormal escape of fluid take place. The occurrence of an abnormal escape of fluid is readily appreciable in many cases, specially where the heating is effected by a condensation of the sun's rays on the axes. In all cases, whatever the nature of the procedure be, the primary effect of the application of heat is a discolouration of the epidermal tissue, which loses more or less the reddish tinge normal to it and becomes greener than the surrounding parts. In many cases where the sun's rays are the disturbing agent, the change, however, manifestly does not cease with this, as a conspicuous exudation of fluid, sometimes to the extent of forming a large drop, takes place, and a similar phenomenon occasionally manifests itself where the heating is effected otherwise. The tissue appears then to undergo such an alteration under the influence of heating as no longer to present sufficient resistance to the pressure of the fluids within it,—a pressure which is normally excessive, as the result of section or puncture of the axes clearly demonstrates.

The question remains how far the phenomenon indicates positive depression of resistance on the part of the tissue or relative depression due to local increase in pressure connected with obstruction? That such obstruction is present there can, I think, be little doubt, for the expansive effect of the elevation of temperature on the gaseous contents of the tissues must almost inevitably give rise to it in greater or less degree. As, however, a visible tendency to abnormal exudation manifests itself in minor degree in some cases of the application of heat to distal parts, such as the tips of distal pinnules, it appears probable that both agencies come into play in various degrees to give rise to the phenomenon in the axes.

But if obstruction and abnormal facilities for the escape of fluid be present in varying degree at a given point in an axis, what are the results which must obtain in any set of appendages such as the leaves which are maintained in their diurnal position by fluid pressure? Clearly they must lie in a tendency to assume the position characteristic of diminished fluid tension in all those areas where fluctuation of a negative character takes place. Now, in any case fluctuation of a negative character must take place distally, whether mere obstruction or mere abnormal escape or both together, are present, but this will not be the case on the central side of the site of development of the abnormal condition. Here the nature of the fluctuation will vary with the relations which conditions causing obstruction hold to those giving rise to facility for escape. Where the former conditions are present in relatively greater degree, the

fluctuations will be positive; where the latter prevail, negative, and, consequently, the effect on the leaves will not be constant as in the previous case, but in one case there will be an increased tendency to the maintenance of the diurnal position, in the latter a tendency to the assumption of the nocturnal one.

According to this the tendency to centrifugal propagation of movement ought to be greater than that to centripetal propagation, and this is precisely what we find to be the case as a matter of experiment. In the case of section of the tips of axes the conditions are different. Here we have to deal solely with conditions favouring abnormal escape of fluid,—conditions under the influence of which negative fluctuations are liable to be propagated centripetally much more constantly and in much greater degree than when conditions of obstruction, too, are present, and, in accordance with this, we find that much more conspicuous and extensive centripetal propagation of movement occurs in many instances in cases of section of the tips of axes than ever present themselves as the result of heating them in their course. There can, I believe, then, be little doubt that the theory which ascribes the propagation of movement to fluctuations in fluid tension is here, again, more in accordance with fact than that which regards it as due to a propagation of stimulation along the course of continuous protoplasm.

We have now to enquire into the evidence for the final proposition regarding the order of events manifested in many cases of propagation of movement. This is contained in Table XII, showing the results following the section of distal pinnules of young heavy leaves heavily loaded with adherent moisture. It shows that in 8 out of the 10 cases dealt with the primary pulvinus was either the very first part of the leaf to act or acted after the injured pinnules alone had moved. The number of experiments included in the table is small, but the results may be taken as typical for those in experiments conducted under like conditions. That this is so is rendered clear by the following data regarding the results of experiments carried out in regard to this point on three distinct mornings. On the first occasion, out of 204 leaves, 135 manifested such premature action in the primary pulvinus; on the second, out of 139 leaves 100 did so; on the third, out of 126 leaves 100 did so. The phenomenon to be accounted for by the theories of the causation of propagation is the discontinuity of action in such cases. According to the protoplasmic theory an explanation can only be arrived at by the assumption of special irritability or contractility in the protoplasm of the primary pulvinus, as compared with that in the other contractile organs. This special irritability or contractility must, moreover, be present in certain cases only, being very frequently present in young and moist leaves, and comparatively rare in old and dry ones, for the differences in the prevalence of the phenomenon in leaves differing in age and amount of adherent moisture is very conspicuous. On the same mornings on which premature pulvinar action occurred in 135 of 204 and in 100 of 139 young leaves loaded with adherent moisture, it only occurred in 11 out of 44 and 7 out of 61 old and

dry leaves. There is, however, nothing to show us that any such special irritability or contractility in the protoplasm of the main pulvinus exists at all or is specially related toage and moisture of the leaves.

When we come to consider the other theory we find that it can afford an explanation both of the occurrence of premature pulvinar action generally and of the greater tendency to such action in young and moist leaves than in old and dry ones. The primary pulvinus has more to contend against in maintaining the expanded condition than any other part of the leaf. The masses of tissue in it determining erection of the leaf have not merely to overcome the opposing thrust of those favouring depression, but have to overcome the downward leverage of the heavy pinnæ situated at the distal extremity of the long petiole. In the case of the secondary pulvini, which deal with lateral movements mainly, the resistance to be overcome is merely that of the opposed masses of tissue, in that of the tertiary pulvini, the action of the masses of tissue favouring expansion is aided by, not opposed to, the action of gravity. But under these circumstances the primary pulvinus must, under certain conditions, be liable to be affected by negative fluctuations in fluid tension incapable of giving rise to conspicuous effects elsewhere; or, in other words, the primary pulvinus, under certain conditions, is adapted to play the part of the most sensitive registering apparatus of the occurrence of fluctuations in the fluid tension of the tissues. Any conditions under which the thrust of the depressant pulvinar tissues is relatively excessive or the weight of the pinnæ abnormally great must tend to increase the sensitiveness of the apparatus. But in young and moist leaves such conditions are present in high degree. In the leaves of *Mimosa pudica* the tissue of the under-half of the pulvinus is relatively weakest when the leaves are young and becomes gradually stronger and more resistant with increasing age. So much is this the case in certain instances that it is not uncommon to meet with old but otherwise healthy leaves in which the primary pulvinus is rigid and petiolar depression accordingly impossible. This gradual increase in relative strength in the structurally weaker masses of pulvinar tissue is not peculiar to *Mimosa pudica.* Much more conspicuous examples of it are afforded by other plants, such as various species of *Bauhinia* and by *Albizzia Lebbek*, in which the young leaves exhibit highly-developed nyctitropic movements, which gradually diminish and ultimately absolutely disappear with increasing age and thickening and rigidity of the primarily passively weaker portions of the pulvinar tissues. The existence of such a change in the relative strength of the opposed masses of tissue accounts, then, for the greater prevalence of premature pulvinar movement in old than in young leaves, and the action of adherent moisture in increasing leverage accounts for its greater prevalence in wet than in dry ones.

The phenomenon of premature pulvinar action is not the only instance of discontinuity in propagation of movement presenting itself to observation.

Other examples frequently occur both in individual leaves and along the course of axes. Examples of such occurrence are contained in several of the tables ; for example, in cases 91 and 92 of Table I, we have examples of discontinuous order in the action of the pinnules of individual pinnæ, in cases 6 and 8 of Table VII we have action in secondarily affected leaves commencing in the pinnæ before any action had manifested itself in the primary pulvinus, in cases 5 and 6 we have discontinuous action along the axis as the result of the application of heat, and in Table XI we have numerous examples of a similar phenomenon as the result of section of the extremities of axes. Especially in these latter cases we see that in certain cases we have to deal, not merely with discontinuity in order of occurrence of action, but with absolute discontinuity of action due to certain intermediate parts failing to act at all when others both distal and proximal to them have acted in greater or less degree. A closely related phenomenon frequently presents itself to observation under certain circumstances in cases of heating axes or section of the extremities of axes where spreading action extends along the axes for a considerable distance but is confined to the primary pulvini alone. In such cases the limitation of action to the primary pulvini is readily explicable as the result of the relatively excessive strain there already alluded to, and the same holds good in regard to the numerous instances in which section of distal pinnules is followed by action confined to the injured pinna and the primary pulvinus. The cases of pure discontinuity in action in the pinnules of a pinna or the leaves on an axis are more difficult to explain, but, in the light of the facts which we have just been considering, it appears certainly more justifiable to ascribe the phenomena to differences of structural power, or in amount of strain in the different parts, than to totally inexplicable and undemonstrated differences in conductivity, irritability, or contractility in the protoplasm.

In dealing with the subject of discontinuity in action there is one thing more which calls for special note, and that is the frequency with which the secondary pulvini fail to act even where complete action has taken place throughout the whole of the rest of a leaf. This is specially the case in cases of action following section of the pinnules, for in such cases, under the conditions in which the experiments dealt with here were conducted, action in the secondary pulvini occurs so infrequently as to be almost exceptional. In cases of action due to the application of heat to pinnules, action in the secondary pulvini is generally well pronounced, but even here exceptions frequently present themselves. This is not the only difference in the phenomena attending heating as compared with section of the pinnules. A reference to the tables will show that premature action in the primary pulvinus is relatively very rare in experiments on heating, and that there is a distinct tendency to wider propagation of action in them than in cases of section. The phenomena of recovery, too, are different according to the exciting cause. In

cases of heating of the pinnules elevation of the petiole is the primary phenomenon of recovery; in cases of section expansion of the pinnules occurs first, and the pinnæ are more or less fully expanded long before the petiole has regained its erect condition, and the same order is followed in recovery after pure mechanically induced movement of the leaves. The precise causation of these differences is a subject calling for further investigation, and I would merely, in the meantime, suggest that it is probably to be found in variations in the amount of fluid discharged from the tissues in the different classes of cases, and in variations in rate of discharge, the phenomena in cases of section corresponding with the occurrence of rapid escape of a limited amount of fluid, those in cases of heating with slow, protracted escape of larger quantities.

The theses originally propounded having now all been considered in detail, and the evidence appearing to support them having been adduced, I have merely to add a few words in regard to a question which has more and more forced itself upon me in the course of working at the subject of the propagation of movement in *Mimosa pudica*. This question is—how far are the movements themselves due to anything more than purely mechanical agency; how far does any active contraction of protoplasm come into play to give rise to them; and how far are they not mere results of fluctuations in the fluid tension of the tissues? The data contained in Table XIII are very suggestive of questions of this nature. In the experiments detailed in this table the sun's rays were carefully focussed on a series of primary pulvini, the application being, as far as possible, confined to the upper surfaces. The 15 experiments registered were conducted on two successive mornings, but numerous other experiments at other periods have demonstrated that the results then obtained were not exceptional. In 8 cases of the 15, the primary phenomenon following the application was elevation of the petiole; in one case it was a rotatory movement; in only 6 was it depression. In the majority of cases in which elevation occurred it was followed by depression, but in two cases the elevation was not followed by depression, although it was followed by action in the pinnæ.

These phenomena of elevation are clear indices of the occurrence of loss of fluid so localised, at the outset at all events, as to cause relative weakening in the tissues of the upper half of the thickness of the primary pulvinus; that is weakening in the tissue directly affected, and, as this was the tissue which makes for depression, to lead to increased elevation of the petiole. The elevation was, as a rule, transitory and soon replaced by depression, because, as the loss of fluid continued, it extended to the tissues on the underside of the pulvinus and thereby re-established the normal potential relations of the opposed masses of tissue, the inferior and structurally weaker becoming naturally absolutely the weaker when loss of fluid had extended to it also. Such a phenomenon is, at all events, well calculated to raise a doubt as to whether the so-called irritable areas in the leaves are really specially irritable, or whether

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they are not merely areas in which disturbances in the fluid tension of the tissues have special facility in giving rise to movements due to structural peculiarities and to relations to other masses of tissue. The areas of special irritability are ordinarily affirmed to lie in the under surface of the primary pulvinus, the inner surfaces of the secondary ones, and the upper surfaces of the pinnules and tertiary pulvini. But these are all areas in which structural peculiarities of the tissues and the mutual relations of various parts must inevitably favour the occurrence of movements as the result of fluctuations in fluid tension incapable of giving rise to movement in other parts in which such adjuvant agents are absent or are replaced by positively opposing ones, as they are in the upper portions of the primary pulvinus, in the outer parts of the secondary ones and in the under parts of the tertiary ones. Where negative fluctuations in fluid tension take place in the so-called irritable areas, they act in favour of the structurally stronger masses of tissue which are constantly striving to establish the passive or nocturnal position of the various parts; whereas in the other areas they act in direct opposition to them. The tendency to more certain and ready occurrence of movement as the result of influences calculated to give rise to negative fluctuations in the fluid content of the tissues, when acting on the so-called irritable areas than when acting elsewhere, can be accounted for on purely physical grounds, and does not necessarily imply the presence of any special irritability or contractility of the protoplasm of the parts. According to the theory that the occurrence of movements is due to mere physical causes, we can account for the distribution of so-called irritability. According to the theory that it is due to special functional properties in the protoplasm of the various masses of tissue, we cannot do so save by the aid of pure assumption.

Whether all the movements which occur in *Mimosa* ought to be regarded as mere mechanical results of fluctuations in the fluid tension, as the nyctitropic ones unequivocally are, remains an open question, but there are grounds for the belief that to a great extent, at all events, they are so. It appears probable that to a very great extent the so-called movements of irritation differ merely in degree, and not in kind, from common nyctitropic ones, the difference lying merely in the degree of rapidity with which the movements are carried out. In the case of the leaves of *Mimosa pudica*, just as in the case of leaves in which all conspicuous movements are of a purely nyctitropic character, the displacement of the various parts is due to the opposition of masses of tissue differing from one another in their structural strength and power of absorption of fluid, those which possess the greatest passive structural strength having the least capacity for absorption and *vice versâ*. Due to this the relative strength of the various masses differs at different times under the influence of different conditions, and corresponding changes take place in the position of the parts on which they work. During the night there is a general diminution in

the fluid content of the tissues and hence the structural stronger masses, although absolutely weaker then than during the day become relatively stronger, and therefore the nocturnal position of parts is always more or less that which they make for. Under the influence of light the structurally weaker masses attain their maximum of relative strength, because of the greater absorption of fluid which they are capable of due to the larger amount of active elements, and specially of chlorophyll containing elements which is present in them than in the opposed masses, and which determines excessive absorption either as a direct result of light stimulation, or as the result of the development of specially absorptive matters by the protoplasm under the influence of such stimulation. The position of the various parts is at any time one due to equilibrium between the opposing masses of tissue, and movements result whenever this equilibrium is disturbed by alterations in their fluid tension. In ordinary nyctitropic leaves facilities are not provided for the occurrence of rapid fluctuations of fluid tension in the tissues, and hence the movements are normally of a slow and insensible character only manifesting themselves under the prolonged influence of condition determining general changes in amount of fluid content of the entire plant. In the case of *Mimosa* the structural peculiarities in the opposing masses of tissue determining the position of the various parts are such as to permit of very rapid redistribution of large masses of fluid, and hence a possibility arises for the occurrence, not merely of movements of the ordinary nyctitropic type, but of rapid movements connected with fluctuations in fluid-content too slight and transitory to be capable of producing appreciable effects apart from such facilities. The equilibrium between the opposing masses of tissue in *Mimosa* is much more unstable than it is in common nyctitropic tissues due to the presence of structural peculiarities, and in connection with this we find corresponding differences in the character of the movements. The instability of diurnal equilibrium in *Mimosa* is further increased by the exceptionally high fluid tension, throughout the plant, which is maintained, in spite of the constant loss of large quantities of fluid, by the absorption of equally large quantities by the roots. In other words, the diurnal condition of the leaves in *Mimosa pudica* is determined by the equilibrium of opposing masses of tissue. But this equilibrium is extremely unstable, as it is only maintained as the result of extreme fluid tension in masses of tissue of very different structural power, in a system from which constant losses of fluid are taking place and in which the structural peculiarities of the parts directly concerned in maintaining the expansion of the leaves afford special facilities for the occurrence of rapid local redistribution of fluid. Under such circumstances we should expect to find that fluctuations in fluid tension incapable of giving rise to conspicuous movements in common nyctitropic leaves are capable of doing so here, and this we find to be actually the case. In fine, the position of expansion of the leaves of *Mimosa*

*pudica* is one of exceedingly unstable equilibrium dependent on the opposed tendencies of different masses of tissue which tend in one case to establish the nocturnal, in the other the diurnal, position of the various parts. The excessive instability of the equilibrium is due, first, to the extreme fluid tension on which it depends, and which is only maintained so long as there is a constant supply of fresh fluid added to the tissues to make good the constant losses due to excessive transudation, and, second, to the presence of structural differences in the opposed tissues specially facilitating the occurrence of diminutions in fluid tension in those which make for the diurnal position. The conditions are such that very slight fluctuations in fluid tension are necessarily adapted to give rise to extensive displacements of the various parts of the leaves, so that it appears doubtful whether it be necessary in any case to assume the occurrence of any active protoplasmic contraction in order to account for the motorial phenomena.

CALCUTTA;
*The 14th April 1887.*

## TABLES.

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica.*

| No. of Experiment. | Result. |
|---|---|
| 1 | Slow and irregular action in the other pinnules of the injured distal pinna; partial and irregular action in the other distal pinna; no further result. |
| 2 | Spreading action in the injured distal pinna; action in its pulvinus, and immediate action in the primary pulvinus; centrifugal action in the pinnules of the proximal pinna of that side of the leaf; no further result. |
| 3 | Spreading action in the pinnules of the cut distal pinna; action in the other distal pinna; action in the proximal pinna of the injured side of the leaf; no further result; no action in the secondary pulvini. |
| 4 | Spreading action in the pinnules of the injured distal pinna; action in one proximal secondary pulvinus; action in the primary pulvinus; no further result. |
| 5 | Commencing action in the pinnules of the injured distal pinna; action in the primary pulvinus; completed centripetal action in the injured pinna; partial action in the other distal pinna and in the proximal pinna of the injured side; no further result. |
| 6 | Results as in No. 5. |
| 7 | Spreading action in the pinnules of the injured distal pinna; commencing action in the pinnules of the proximal pinna of the injured side of the leaf; action in the primary pulvinus, and commencing action in the opposite distal pinna; action of the other proximal pinna; no further result. |
| 8 | Results as in No. 7. |
| 9 | Partial spread of action along the injured proximal pinna; action in the primary pulvinus, and commencing action in the distal pinna of the injured side; partial action in the other distal pinna; no further result. |
| | *The above experiments were carried out on various occasions during the rains.* |
| 10 | *Leaf not yet reached by the sunshine.*—Very slow and incomplete action in the cut pinna, the pinnules of the outer third and those quite at the base acting imperfectly, whilst an intermediate groups of four pinnules on either side failed to move; no further result. |

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica*—continued.

| No. of Experiment. | Result. |
|---|---|
| 11 | *Leaf not yet reached by the sunshine.*—Very slow and partial action in the pinnules of the distal half of the pinna, and in one on one side of the middle of the proximal half ; no further result. |
| 12 | *Leaf not yet reached by the sunshine ; section involving the tip of the secondary petiole.*—Rapid, progressive action in the pinnules of the cut pinna, followed by immediate action in the primary pulvinus ; no further result. |
| 13 | *Leaf and section as in No. 12.*—Immediate action of about six pairs of distal pinnules and of the primary pulvinus ; pause ; very slow, progressive action in the other pinnules of the distal half of the cut pinna ; no further result. |
| 14 | *Leaf not yet reached by the sunshine ; section involving tips of distal pinnules only.*—Very slow and partial action in the pinnules of the distal third of the cut pinna, and in some pinnules on one side at the base ; no further result. |
| 15 | *Leaf not yet reached by the sunshine ; section involving the tip of the secondary petiole.*—Imperfect action in all the pinnules of the cut pinna ; no further result. |
| 16 | *Leaf in full sunshine ; section involving tips of pinnules only.*—Spreading action in the pinnules of the cut pinna ; action in the primary pulvinus ; complete action in the pinnules of the other pinnæ ; no further result. |
| 17 | *Leaf in full sunshine ; section involving tip of secondary petiole.*—Action in the pinnules of the cut pinna ; pause ; action in the secondary and primary pulvini ; complete action in the remaining pinnæ ; action in the primary pulvinus of the first leaf above. |
| 18 | *Leaf and section as in No. 17.*—Spreading action in the pinnules of the cut pinna ; action in the primary pulvinus ; complete action in the other pinnæ ; no further result. |
| 19 | *Leaf in full sunshine ; section involving pinnules only.*—Spreading action in the pinnules of the cut pinna ; action in the primary pulvinus ; complete action in the remaining pinnæ ; nothing more. |
| 20 | *Leaf and section as in No. 17.*—Spreading action in the pinnules of the cut pinna ; pause ; action in the primary pulvinus ; complete action in the other pinnæ ; no further result. |
| 21 | *Leaf and section as in No. 17.*—Spreading action in the pinnules of the cut pinna ; pause ; action in the primary pulvinus ; complete action in the other pinnæ ; no further result. |

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica*—continued.

| No. of Experiment. | Result. |
|---|---|
| 22 | *Leaf in full sunshine; section involving pinnules only.*—Spreading action in the pinnules of the cut pinna; action in the secondary pulvinus, and commencing action in the pinnules of the distal pinna of the same side of the leaf; action in the primary pulvinus; complete action in the remaining pinnæ; action in the primary pulvinus of the next leaf above; complete action in three of its pinnæ, and incomplete action in the fourth one; no further result. |
| | *Experiments 10 to 22 were all conducted at an early hour on one morning, and very clearly show the effect of protection from, or exposure to, conditions favouring loss of fluid by evaporation. The morning was that of the 30th December 1886. It was mild and hazy, quite still, and with a drenching dew.* |
| 23 | *Leaf not yet reached by the sunshine; section involving tips of pinnules only.*—Action in the first two or three pairs of pinnules; action in the primary pulvinus; progressive, slow, centripetal action in the pinnules of the cut pinna; imperfect action in the pinnules of the other pinnæ; no further result. |
| 24 | *Leaf not yet reached by the sunshine; section involving the tip of the secondary petiole.*—Spreading, centripetal action in the pinnules of the cut pinna; action in the primary pulvinus; imperfect action in the pinnules of the remaining pinnæ. |
| 25 | *Leaf exposed to direct sunshine; section involving the tips of the pinnules only.*—Complete, spreading action in that leaf and in the leaf next above. |
| 26 | *Leaf and section as in No. 25.*—Complete, spreading action in that leaf; no further result. |
| 27 | *Leaf and section as in No. 25.*—Complete, spreading action in that leaf, in one below, and in one above it. |
| 28 | *Leaf and section as in No. 25.*—Complete, spreading action in that leaf and in one leaf below it. |
| 29 | *Leaf exposed to direct sunshine; section involving the tip of the secondary petiole.*—Complete, spreading action in that leaf; no further result. |
| 30 | *Section involving the tip of the secondary petiole.*—Complete action in the leaf; no further results. |
| 31 | Section and results as in the previous case. |
| 32 | *A two-pinnaed leaf; section involving the tip of the secondary petiole.*—Complete, spreading action in that pinna; nearly complete action in the other one; no further result. |

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica*—continued.

| No. of Experiment. | Result. |
|---|---|
| 33 | *Leaf not yet reached by the sunshine.*—Action of two or three pairs of pinnules; action in primary pulvinus; slow, partial, centripetal action in the cut pinna; slow, centrifugal action in the rest of the pinnæ; no further result. |
| 34 | *Leaf in shade.*—Very slow, complete, centripetal action in the cut pinna; action in the primary pulvinus when the action in the cut pinna had almost reached the base; very slow, partial action in the other pinnæ; no further result. |
| 35 | *Leaf exposed to direct sunshine.*—Complete, spreading action in the cut leaf; partial action in one beneath; complete action in the first leaf above; action in the primary pulvinus of the third leaf above; very slow, partial action in its pinnæ; no further results. |
| 36 | *Leaf exposed to direct sunshine.*—Complete, spreading action in the cut leaf; no further result. |
| 37 | *Leaf exposed to direct sunshine.*—Result as in the previous case. |
| 38 | *Ditto* Ditto. |
| 39 | *Ditto* Ditto. |
| 40 | *Leaf in shade.*—Partial action in the cut pinna; slight action in the primary pulvinus; no further result. |
| 41 | *Leaf in shade.*—Action in the distal two-thirds of the cut pinna; no further result. |
| 42 | *Leaf in shade.*—Action in the cut pinnules; action in the primary pulvinus; slow, progressive, centripetal action in the pinnules in the distal third of the cut pinna; no further result. |
| 43 | *Leaf in shade.*—Slow, progressive, complete action in the cut pinna; no further result. |
| 43 | *Leaf in shade.*—Complete, progressive action in the cut pinna; long pause; action in the primary pulvinus; no further result. |
| 44 | *Leaf in shade.*—Action in the cut pinna; action in the primary pulvinus, and extreme depression; partial action in the remaining pinnæ; no further result. |
| 45 | *Leaf in shade.*—Action in the cut pinna; action in the primary pulvinus; no further result. |

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica*—continued.

| No of Experiment. | Result. |
|---|---|
| 46 | *Leaf exposed to direct sunshine.*—Complete, progressive action in the cut pinna; action in the primary pulvinus; complete action in the remaining pinnæ; no further result. |
| 47 | *Leaf exposed to direct sunshine.*—Results as in the previous case. |
| 48 | *Ditto* Ditto. |
| 49 | *Ditto* Ditto. |
| 50 | *Ditto* Ditto. |
| 51 | *Leaf exposed to direct sunshine.*—Complete action in the cut pinna; commencing action in the other distal pinna; action in the primary pulvinus; complete action in the remaining pinnæ. |
| 52 | *Leaf in shade.*—Partial action in the cut pinna; full action in the primary pulvinus; no further result. |
| 53 | *Leaf exposed to direct sunshine.*—Complete action throughout the entire leaf; no further result. |
| 54 | *Leaf in the shade and exposed to rain.*—Very slow and partial action in the cut pinna; action in the primary pulvinus; no further result. |
| 55 | *Conditions as in the previous case.*—Results as in the previous case. |
| 56 | *Conditions as in No. 54.*—Action in the cut pinnules; action in the primary pulvinus; long pause; very slow and partial action in the pinnules of the distal half of the cut pinna; no further result. |
| 56 | *Conditions as in No. 54.*—Results as in the previous case. |
| 57 | *Conditions as in No. 54; tip of one pinnule only cut.*—Slow, progressive, well-developed action in the pinnules in the distal half of the cut side of the pinna; very feeble, partial action in a few of the distal pinnules of the opposite side of the cut pinna; no further result. |
| 57 | *Conditions as in the previous case.*—Results as in the previous case. |
| 58 | *Leaf exposed to direct sunshine.*—Partial action throughout the entire leaf; no further result. |
| 59 | *Leaf exposed to direct sunshine.*—Complete action throughout the entire leaf; no further result. |

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica*—continued.

| No. of Experiment. | Result. |
|---|---|
| 60 | *Leaf exposed to direct sunshine.*—Complete action throughout the entire leaf; no further result. |
| 61 | *Ditto* ditto ditto. |
| 62 | Slow, gradual action of the entire leaf; no further result. |
| 63 | Partial action in all the pinnæ; no further result. |
| 64 | Almost complete action throughout the entire leaf; no further result. |
| 65 | Partial action in all the pinnæ, and action of the primary pulvinus; no further result. |
| 66 | Almost complete action throughout the entire leaf; no further result. |
| 67 | Action in the cut pinnules; long pause; action in the primary pulvinus, but depression of the petiole mechanically prevented; long pause; slow, partial, centripetal action throughout the cut pinna; no further result. |
| 68 | Action in the cut pinnules, and in one or two pairs immediately beneath them; long pause; action in the primary pulvinus; pause; centripetal action throughout the cut pinna; partial action in the remaining pinnæ; no further result. |
| 69 | *Leaf in the shade.*—Action in the cut pinnules; long pause; action in the primary pulvinus, but depression of the petiole mechanically obstructed; very slight action in two or three pairs of pinnules beneath the cut ones; no further result. |
| 70 | *Leaf exposed to direct sunshine.*—Partial action throughout the entire leaf save the proximal pinna of the uncut side; no further result. |
| 71 | *Leaf exposed to direct sunshine.*—Slow, complete action throughout the entire leaf; no further result. |
| 72 | *Leaf in shade.*—Spreading, incomplete action in all the pinnules of the cut pinna; incomplete action in the basal halves of two other pinnæ; no further result; primary pulvinus responded readily to direct impulse. |
| 73 | *Leaf in shade.*—Incomplete action throughout the distal half of the cut pinna; no further result. |
| 74 | *Leaf in shade.*—Incomplete action throughout the cut pinna; no further result. |
| 75 | *Leaf exposed to direct sunshine.*—Complete action in the leaf; no further result. |

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica*—continued.

| No. of Experiment. | Result. |
|---|---|
| 76 | *Leaf exposed to direct sunshine.*—Complete action throughout the leaf save in the tip of one pinna; depression of the petiole somewhat obstructed mechanically; no further result. |
| 77 | *Leaf exposed to direct sunshine.*—Complete action throughout the leaf save in one pinna; no further result. |
| 78 | *Leaf exposed to direct sunshine.*—Complete action throughout the entire leaf; no further result. |
| 79 | *Ditto* ditto ditto. |
| 80 | *Leaf exposed to direct sunshine.*— Complete action in the cut leaf; no further result. |
| 81 | *Ditto* ditto ditto. |
| 82 | *Ditto* ditto ditto. |
| 83 | *Ditto* ditto ditto. |
| 84 | *Ditto* ditto ditto. |
| 85 | *Ditto* ditto ditto. |
| 86 | *Leaf exposed to direct sunshine.*—Pause; action in three or four pairs of pinnules; action in the primary pulvinus; almost complete action throughout the rest of the leaf; no further result. |
| 87 | *Leaf exposed to direct sunshine.*—Centripetal action throughout the cut pinna; commencing centrifugal action in the other distal pinna; action in the primary pulvinus; complete action throughout the rest of the leaf; no further result. |
| 88 | *Leaf exposed to direct sunshine.*—Very irregular and partial action in that pinna; partial action in the other distal pinna and in the proximal pinna of the cut side of the leaf; no further result; primary pulvinus partially rigid. |
| 89 | *Leaf exposed to direct sunshine.*—Complete action in the cut pinna; pause; complete action in the primary pulvinus, and extreme depression; complete action in the other pinnæ; no further result. |
| 90 | *Leaf exposed to direct sunshine.*—Complete action in the cut pinna; commencing action in the other distal pinna; action in the primary pulvinus; complete action in the rest of the leaf; no further result. |

TABLE I.—*General Table of Results of section of Distal Pinnules of Mimosa pudica*—concluded.

| No. of Experiment. | Result. |
|---|---|
| 91 | *Leaf in shade and plant drenched with dew.*—Pause; centripetal action in six pairs of pinnules; pause; action in the fifth pair of pinnules beneath those which had previously acted; pause; action in the fifth pair below those which had last acted; no further action in the cut pinna or elsewhere; primary pulvinus readily responsive to direct impulse. |
| 92 | *Conditions as in No. 91.*—Pause; action in seven successive pairs of pinnules; partial action in the pinnules at the base of the pinna; spread of centripetal action along the distal part, but action not extending to the area of basal action; action of the primary pulvinus, and extreme depression; action in one or two pinnules on one side of the base of the other distal pinna; no further result. |
| 93 | *Conditions as in No. 91.*—Very slow and very incomplete action in the cut pinna; action in the primary pulvinus, and extreme depression; no further result. |
| 94 | *Conditions as in No. 91.*—Very slow action in the tip of the cut pinna; very slow action in the basal portion; slow, centripetal action in the central portion; action in the primary pulvinus, and extreme depression; no further result. |
| 95 | *Conditions as in No. 91.*—Results as in No. 94 save that several of the central pinnules of the cut pinna failed to act. |
| 96 | *Conditions as in No. 91.*—Action in the cut pinnules; pause; slight action in one or two pinnules at scattered intervals along the course of the cut pinna; no further result; primary pulvinus responding readily to direct impulse. |
| 97 | *Conditions as in No. 91.*—Action in the cut pinnules; action in the primary pulvinus; incomplete centripetal action in the cut pinna; no further result. |
| 98 | *Conditions as in No. 91.*—Fairly complete action throughout the cut pinna save in three pinnules at the base; no further result; primary pulvinus readily responsive to direct impulse. |
| 99 | *Conditions as in No. 91.*—Results as in No. 97. |
| 100 | *Conditions as in No. 91.*—Incomplete action throughout the cut pinna; no further result; primary pulvinus readily responsive to direct impulse. |

A total of one hundred experiments, in only six of which there was any extension of movement beyond the injured leaf.

TABLE II.—*Showing Results of section of Distal Pinnules on mornings after a fall of Rain.*

| No. of Experiment. | Result. |
|---|---|
| | *These experiments were carried out on two successive mornings, those of the 20th and 28th March, a fall of rain having occurred on each of the previous evenings. The brake of the plant was not exposed to direct sunshine at the time of experiment, and the leaves were clean washed but nearly dry, there having been hardly any dew during the preceding nights. All the leaves were young ones.* |
| 1 | Action in the cut pinnules; pause; action in the primary pulvinus; spreading action in the cut pinna; action in the base of the other distal pinna; long pause; partial and slow action throughout two other pinnæ, and slight action in the base of the third one; no further result; very rapid recovery. |
| 2 | Spreading action in the cut pinna; action in the primary pulvinus, and extreme depression; slow and partial action in the bases of two of the other pinnæ; no further result; very rapid recovery. |
| 3 | Very slow, spreading action in that pinna save in the basal pair of pinnules; no further result. |
| 4 | Very slow, partial action in the pinnules of the cut pinna save three basal ones; no further result. |
| 5 | Action in cut pinnules; pause; action in primary pulvinus; slow, almost complete, centripetal action in the cut pinna; no further result. |
| 6 | Slow, centripetal action in the cut pinna; action in the primary pulvinus, and extreme depression; slow, partial action throughout one other pinna; and very slow, very partial action in the basal two-thirds of another; no further result. |
| 7 | Action in the cut pinnules; and in one or two pairs beneath them; pause; action in the primary pulvinus; long pause; spreading centripetal action in the cut pinna; pause; slight action in the base of the other distal pinna; no further result; primary pulvinus readily responsive to mechanical impulse. |
| 8 | *Tip of one pinnule only cut.*—Action in that pair; pause; action in primary pulvinus, and full depression; long pause; very slow, partial action in the base of the other pinna (a two pinnaed leaf); no further result. |
| 9 | Action in the cut pinnules; pause; action in the primary pulvinus; very slow action in the pinnules of the cut pinna; slow, partial action in the other pinna (a two pinnaed leaf) commencing ere action had extended to the base of the cut one. |
| 10 | Slow, almost complete action in that pinna; pause; action in the primary pulvinus, and extreme depression; slow, partial action in two other pinnæ; no further result. |

TABLE II.—*Showing Results of section of Distal Pinnules on mornings after a fall of Rain*—continued.

| No. of Experiment. | Result. |
| --- | --- |
| 11 | Slow, spreading, complete action in the cut pinna; action in the primary pulvinus, and full depression; slow, incomplete action in two other pinnæ; long pause; very incomplete action in the base of the remaining pinna. |
| 12 | *Tip of one pinnule only cut.*—Action in that pair of pinnules; pause; action in the primary pulvinus, but depression only partial due to mechanical obstruction; pause; centripetal action in the outer half of the cut pinna; no futher result. |
| 13 | Action in the cut pinnules; long pause; very slow but almost complete action in that pinna; no further result; primary pulvinus readily responsive to direct impulse. |
| 14 | Results as in the previous case. After an interval, but before the first pinna had completely recovered, the distal pinnules of the other pinna (a two-pinnaed leaf) cut with the following results:—Pause; action in the primary pulvinus, and full depression; complete action in that pinna; no further result. |
| 15 | Action in the cut pinnules; action in the primary pulvinus, and full depression; complete centripetal action in the cut pinna; no further result. |
| 16 | Action in the cut pinnules; pause; action in the primary pulvinus, but depression incomplete due to mechanical obstruction; pause; complete, centripetal action in the cut pinna; no further results. |
| 17 | Pause; slow, spreading action in that pinna; pause; action in the primary pulvinus, and extreme depression; spreading centrifugal action in two other pinnæ, complete almost to the tips; no further result. |
| 18 | Action in the cut pinnules; pause; slow, centripetal action in the cut pinna; pause; conspicuous action in its secondary pulvinus; pause; action in the primary pulvinus, and almost full depression; imperfect centrifugal action almost to the tip of the other distal pinna; pause; slow, incomplete action in the base of the proximal pinna of the same side of the leaf as the cut pinna; no further result. |
| 19 | Long pause; very slow action in the cut pinnules; very slow, centripetal action in the distal half of the cut pinna; action in the primary pulvinus, but incomplete depression due to obstruction; pause; action of all the remaining pinnules on one side of the cut pinna save the two basal ones; no action in the six lowest pinnules of the other side; no further result. |
| 20 | Action in the cut pinnules; pause; rapid, complete centripetal action throughout the cut pinna; no further result. After an interval, and when the first pinna had fully expanded in its basal half, the tip of the pinnules of the other distal pinna were cut with the following results:—Action in the cut pinnules; pause; action in the primary pulvinus and full depression; pause; complete, centripetal action in the second pinna; pause; almost complete, centrifugal action in the expanded, basal portion of the first pinna; no further result. |

The results of the experiments detailed in the present table are specially

significant when compared with those in the succeeding one. There is a general resemblance in the phenomena in both, just as there was a general resemblance in the conditions to which the plants were subject at the time of experiment. There are at the same time differences in details of action corresponding with details in the external conditions. When the experiments included in Table III were carried out, the amount of soil moisture and of water on the leaves was much greater than when those of the present table were conducted, and coinciding with this we find indications of diminished tendency to extension of action, and increased tendency to early action in the primary pulvinus. This will appear more clearly if the results in these respects are tabulated alone :—

*Table II.*—20 experiments.
Action extending to other pinnæ in 10 cases.
Premature action in the primary pulvinus in 8 cases.

*Table III.*—10 experiments.
Action extending to other pinnæ in 4 cases.
Premature action in the primary pulvinus in 7 cases.

The conditions present in the experiments of Table III implied greater supply of moisture and greater mechanical strain on the primary pulvinus than those present in the experiments of Table II, and these were accompanied by more localised action and a greater tendency to premature action in the primary pulvinus.

---

TABLE III.—*Showing Results of section of Distal Pinnules after a continuous period of heavy Rainfall.*

| No. of Experiment. | Result. |
|---|---|
| | *The experiments detailed in this table were conducted between 8 and 9 A.M. of the 30th March after a night of continued heavy rain. The morning was very cloudy, so that the brake of the plant had not been exposed to direct sunshine. No rain was falling at the time, but the leaves, which were widely expanded, were, as a rule, heavily loaded with drops of water. All the leaves experimented with were fully developed but young ones, with four pinnæ.* |
| 1 | Action in the cut pinnules; pause; action in the primary pulvinus; long pause; very slow, partial action to near the base of the cut pinna; no further result. |
| 2 | Action in the cut pinnules; long pause; very slow and partial, centripetal action in the cut pinna in its outer three-fourths; action in the primary pulvinus, but depression mechanically partially obstructed; pause; centripetal, imperfect action in the pinnules of one side of the basal fourth of the cut pinna: no further result. |

TABLE III.—*Showing Results of section of Distal Pinnules after a continuous period of heavy Rainfall*—continued.

| No. of Experiment. | Result. |
|---|---|
| 3 | Action in the cut pinnules; long pause; action in the primary pulvinus; long pause; very slow, imperfect action in the distal two-thirds of the cut pinna; no further result. |
| 4 | Action in the cut pinnules; long pause; very slow, incomplete action in the distal two-thirds of the cut pinna; pause; action in one pinnule below; no further result. |
| 5 | Action in the cut pinnules; long pause; action in the primary pulvinus; pause; slow, incomplete action in the distal three-fourths of the cut pinna; no further result. |
| 6 | Action in the cut pinnules; long pause; action in the primary pulvinus, but depression somewhat obstructed; slow, incomplete, centripetal action throughout the cut pinna, and partial, very imperfect centrifugal action in one other pinna. |
| 7 | Action in the cut pinnules; long pause; action in the primary pulvinus; almost complete action in the cut pinna; slight, very imperfect action in one other pinna a little above the base. |
| 8 | Action in the cut pinnules; pause; action in the primary pulvinus, and full depression; long pause; very slow, imperfect action in the distal three-fourths of the cut pinna; long pause; imperfect action of the basal pinnules of one side of the cut pinna; no further result. |
| 9 | Action in the cut pinnules; long pause; action in the primary pulvinus; long pause; very slow, very imperfect action in the cut pinna and in one other pinna; no further result. |
| 10 | Action in the cut pinnules; long pause; very slow and exceedingly imperfect action in the distal two-thirds of the cut pinna; slow action in the primary pulvinus, and full depression; very imperfect action in the basal third of the cut pinna, and very slow, very imperfect action in one other pinna; no further result. |

The phenomena presented in this series of experiments are just what ought to have occurred according to the view that alterations in fluid tension, and the degree of strain present in various parts in maintaining the expanded condition, are the essential determinants of the propagation of movements from one part to another of the plant. The plants were very moist, the supply of root moisture excessive, the atmospheric humidity high, and the leaves loaded with adherent water. Coinciding with these conditions there was very slow, imperfect, and limited action, and a great tendency to early action on the part of the primary pulvinus, action in it occurring in 7 out of the 10 cases as the first phenomenon after action in the cut pinnules.

*TABLE IV.—Showing the Results following section of Pinnules at different points in Pinnæ.*

| No. of Experiment. | Result. |
|---|---|
| | *A.—Section of tips of distal pinnules of distal pinnæ.* |
| 1 | Action in that pinna ; slight action in its secondary pulvinus ; action in the primary pulvinus, but depression somewhat obstructed mechanically ; no further result. |
| 2 | Action in that pinna ; no further result. |
| 3 | Pause ; action in the primary pulvinus ; centripetal action in that pinna ; centrifugal action almost to the tip of the other distal pinna ; no further result. |
| 4 | Slow action in that pinna, imperfect towards the base ; action in the primary pulvinus ; slight action in the bases of two other pinnæ ; no further result. |
| 5 | Progressive, complete action in that pinna ; no further result ; primary pulvinus readily responsive to mechanical impulse ; re-expansion very rapid. |
| 6 | Progessive, complete action to nearly the base of that pinna ; no further result ; primary pulvinus readily responsive to mechanical impulse. |
| 7 | Pause ; full action in the primary pulvinus ; spreading, complete, centripetal action in that pinna ; no further result. |
| 8 | Very slow and imperfect action in that pinna; no further result; primary pulvinus readily responsive to mechanical impulse. |
| 9 | Pause ; action in the primary pulvinus ; action in the cut pinna ; no further result. |
| 10 | Slow and almost complete action in that pinna ; pause ; action in the primary pulvinus ; no further result. |
| | *B.—Section of tips of proximal pinnules of proximal pinnæ.* |
| 11 | Pause ; action in the primary pulvinus ; slow, slight, imperfect action in the bases of both proximal pinnæ ; no further result. |
| 12 | Pause ; commencing centrifugal action in the base of that pinna ; action in the primary pulvinus ; completed centrifugal action in that pinna ; incomplete action in the base of two, and complete action in the base of the third remaining pinna. |
| 13 | Pause ; incomplete action in the base of that pinna ; incomplete action in the basal halves of the other pinnæ ; no further result ; primary pulvinus readily responsive to mechanical impulse. |

TABLE IV.—*Showing the Results following section of Pinnules at different points in Pinnæ*—continued.

| No. of Experiment. | Result. |
|---|---|
| 14 | Commencing centrifugal action in that pinna; action in the primary pulvinus; completed action in the cut pinna extending nearly to the tip; long pause; centrifugal action almost to the tip of the remaining pinna (a two-pinnaed leaf). |
| 15 | Pause; action in that and the opposite secondary pulvinus; slow, spreading action in the bases of both proximal pinnæ; pause; slight action in the bases of both distal pinnæ; no further result; primary pulvinus rigid. |
| 16 | Long pause; slow, centrifugal action in the base of that pinna; pause; action extended almost to the tip; slow action in the basal half of the remaining pinna (a two-pinnaed leaf); no further result; primary pulvinus readily responsive to mechanical impulse. |
| 17 | Pause; very slow, imperfect action in the basal two-thirds of both pinnæ (a two-pinnaed leaf); no further result; primary pulvinus rigid. |
| 18 | Pause; slow, centrifugal action in that and the other proximal pinna; pause action in the primary pulvinus; slow, partial action in the other two pinnæ. |
| 19 | Slow, partial, imperfect action in that pinna; action in the primary pulvinus; slow, partial action in the remaining pinnæ. |
| 20 | Action in the primary pulvinus; imperfect action in that pinna; long pause; partial action in the basal two-thirds of the other pinnæ. |
| | *C.—Section of tips of central pinnules of proximal pinnæ.* |
| 21 | Pause; imperfect centripetal action in that pinna; pause; very imperfect centrifugal action in it; no further result; the primary pulvinus readily responsive to mechanical impulse. |
| 22 | Pause; centripetal action in that pinna; very imperfect centrifugal action in it; very slow, imperfect action in the basal halves of the remaining pinnæ; no further result; primary pulvinus readily responsive to mechanical impulse. |
| 23 | Pause; imperfect centripetal action; pause; very slow, imperfect centrifugal action; action in the primary pulvinus; imperfect action in the remaining pinnæ; no further result. |
| 24 | Pause; centripetal action in that pinna; centrifugal action in it; action in the primary pulvinus; incomplete action in the other pinnæ; no further result. |
| 25 | Action in the primary pulvinus; centripetal action in the cut pinna; very slow, centrifugal action in it; imperfect action in the other pinnæ; no further result. |

TABLE IV.—*Showing the Results following section of Pinnules at different points in Pinnæ*—concluded.

| No. of Experiment. | Result. |
|---|---|
| 26 | Pause ; centripetal action in that pinna ; long pause ; very slow, centrifugal action in it ; action in the primary pulvinus ; slight action in the other pinnæ ; no further result. |
| 27 | Pause ; centripetal action in the cut pinna ; imperfect, centrifugal action in it ; action in the primary pulvinus ; slight action in the remaining pinnæ. |
| 28 | Pause ; centripetal action in the cut pinna ; long pause ; action in its secondary pulvinus ; long pause ; centrifugal action in the cut pinna ; very slight action in the remaining pinnæ ; no further result ; primary pulvinus readily responsive to mechanical impulse. |
| 29 | Pause ; centripetal action in the cut pinna ; centrifugal action in it ; action in the other pinna (a two-pinnaed leaf) ; action in the primary pulvinus ; no further result. |

This table consists of the results in a series of experiments carried out in a morning following a night in the early part of which there had been a heavy shower of rain. The morning was cloudless but slightly hazy, the sunshine had not at the time of experiment reached the brake of *Mimosa*, and the leaves were widely expanded, well-washed but relatively dry, the amount of adherent moisture being trifling as compared with that present after a normal dewy night. The results of the experiments are typical for plants under similar conditions. The amount of action in the leaves was almost in every case very limited, the action sometimes being confined to the cut pinna, sometimes extending to other pinnæ in greater or less degree, but leaving the primary pulvinus unaffected, and in no case was the action complete throughout the leaf. In 6 cases only did the primary pulvinus act immediately in sequence to the cut pinnules, but in 10 cases it acted before any of the uninjured pinnæ and in some of these before action in the cut pinna was completed. These results in regard to the period at which the primary pulvinus acted should be compared with those in Table VII, in which we have 10 cases, in 8 of which action in the primary pulvinus immediately succeeded action in the injured pinnules. This difference, as well as the generally-limited character of the action in the leaves, cannot be ascribed to any incapacity for action in these cases, for the contractile apparatus almost without exception responded fully to direct impulses. They are, however, just what the conditions under which the plants were at the time of experiment will account for. There had been a recent and abundant supply

of moisture, but the leaves were not weighted by it, and the direct sunshine had not yet impinged on the brake. There was an abundant supply of root moisture, slight evaporation, and an absence of extraneous strain on the primary pulvini coincident with very limited extension of action and delay in the action of the primary pulvini.

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TABLE V.—*Results of Experiments on section of Distal Pinnules in Dry Leaves fully exposed to direct sunshine.*

| No. of Experiment. | Result. |
|---|---|
| 1 | Spreading and complete action in that leaf; no further result. |
| 2 | Ditto ditto ditto. |
| 3 | Ditto ditto ditto. |
| 4 | Ditto ditto ditto. |
| 5 | Ditto ditto ditto. |
| 6 | Ditto ditto ditto. |
| 7 | Ditto ditto ditto. |
| 8 | Ditto ditto ditto. |
| 9 | Ditto ditto ditto. |
| 10 | Ditto ditto ditto. |
| 11 | Ditto ditto ditto. |
| 12 | Ditto ditto ditto. |
| 13 | Spreading almost complete action in that leaf; no further result. |
| 14 | Ditto ditto ditto. |
| 15 | Spreading complete action in that leaf; action in the first leaf below. |

The results in this table contrast strongly with those in the preceding one. The difference is, however, quite in accordance with the difference in conditions present in this case. The experiments were here conducted on a bright, clear morning, with very warm sunshine and relatively dry air and after the brake

of the plant had been for some time exposed to strong, direct sunshine. Under such circumstances, there was much more complete and extensive action than under the conditions present in the case of the experiments of the preceding table, the cut leaves acting completely in 13 of the 15 cases, and action extending to a second leaf in one.

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TABLE VI.—*General Table of Results of Application of Heat to the tips of Distal Pinnules.*

| No. of Experiment. | Result. |
|---|---|
| | *A.—Heat applied to tips of distal pinnules of distal pinnæ.* |
| 1 | Spreading, centripetal action in that pinna ; action in the primary pulvinus ; action in the remaining pinnæ ; action in the primary pulvinus alone of the second leaf beneath the heated one ; action in the primary pulvinus of the first leaf below ; action in the primary pulvinus of the first leaf above. |
| 2 | Spreading, centripetal action in the pinnules of that pinna ; commencement of action in the other distal pinna ; action in the primary pulvinus. |
| 3 | Spreading, centripetal action in that pinna ; action in the primary pulvinus ; action in the other distal pinna. |
| 4 | Spreading, centripetal action in that pinna ; conspicuous action in the secondary pulvini ; immediate action of the primary pulvinus ; spreading, centrifugal action in the pinnules of the other pinnæ. |
| 5 | Spreading action in that pinna ; pause ; action in primary pulvinus ; action in secondary pulvini and pinnules of the other pinnæ. |
| 6 | Spreading action in that pinna ; action in the secondary pulvini ; action in the primary pulvinus ; spreading, centrifugal action in the other pinnæ. |
| 7 | Ditto ditto ditto. |
| 8 | Spreading action in that pinna ; long pause ; action in the secondary pulvinus of the proximal pinna of the same side and spreading, centrifugal action of the pinnules ; action of the other distal secondary pulvini and of the primary pulvinus ; spreading, centrifugal action in the pinnules of the second distal pinna ; action in the other proximal pinna. |
| 9 | Spreading action in that pinna ; action in the other distal secondary pulvinus ; action in the primary pulvinus ; action in the remaining secondary pulvini and pinnules. |

TABLE VI.—*General Table of Results of Application of Heat to the tips of Distal Pinnules*—continued.

| No. of Experiment. | Result. |
|---|---|
| 10 | Action in that pinna; strong action in all the secondary pulvini; action in primary pulvinus, and spreading, centrifugal action in the pinnules of the other three pinnæ; action in one leaf below and in two above. |
| 11 | Spreading action in the pinnules of that pinna; action in its secondary pulvinus and in the primary pulvinus; action in the remaining secondary pulvini and pinnules. |
| 12 | Spreading action in the pinnules of that pinna; strong action in all the secondary pulvini; spreading, centrifugal action in the pinnæ of the opposite side of the leaf, and action in the primary pulvinus; action in the proximal pinna of the same side; centrifugal action in the two leaves immediately above. |
| 13 | Action in that pinna; action in the secondary pulvini of the opposite distal pinna and of the proximal pinna of the affected side of the leaf; commencing action in their pinnules, and action in the primary pulvinus; action in the proximal pinna of the opposite side. |
| 14 | Spreading action in that pinna; pause; action in the primary pulvinus; action in the remaining pinnæ. |
| 15 | Spreading action in that pinna; action in the other distal pinna; commencing action in the proximal pinna of that side, and action in the primary pulvinus; action in the other proximal pinnæ. |
| 16 | Spreading action in that pinna; action in the primary pulvinus; action in the remaining pinnæ. |
| 17 | Action in the pinnules of that pinna; action in the secondary pulvini; action of the primary pulvinus, and commencing action in the other distal pinna; action in the proximal pinnæ. |
| 18 | Action in the pinnules of that pinna; action in the secondary pulvinus of that pinna; centrifugal action in the other distal pinna; commencing action in the proximal pinna of the affected side, and action in the primary pulvinus; action in the other proximal pinna. |
| 19 | Spreading action in the pinnules of that pinna; action in secondary and primary pulvini; action in the remaining pinnæ; action in the leaf immediately above. |
| 20 | Spreading action in the pinnules and secondary pulvinus of that pinna; action in the primary pulvinus; action in the other pinnæ. |

TABLE VI.—*General Table of Results of Application of Heat to the tips of Distal Pinnules*—continued.

| No. of Experiment. | Result. |
|---|---|
| | *B.—Heat applied to the tip of a proximal pinna.* |
| 21 | Spreading action in the pinnules of that pinna ; action in its secondary pulvinus ; action in the primary pulvinus, and spreading action in the distal pinna of the affected side ; action in the opposite distal pinna. |
| 22 | Spreading action in the pinnules and secondary pulvinus of that pinna ; commencing action in the distal pinna of that side ; action in the primary pulvinus ; action in the other pinnæ. |
| 23 | Spreading action in the pinnules of that pinna ; action in all the secondary pulvini ; action in the primary pulvinus, and commencing action in the pinnules of the distal pinna of the affected side ; action in the other pinnæ. |
| 24 | Spreading action in the pinnules of that pinna ; action of both secondary pulvini of that side of the leaf ; action in the primary pulvinus ; pause ; action in the pinnules of the distal pinna of the affected side ; action in the other pinnæ. |
| 25 | Spreading action in the pinnules of that pinna ; action in its secondary pulvinus ; action in the secondary pulvinus of the distal pinna of the same side of the leaf, and commencing action in its pinnules ; action in the secondary pulvinus of the other distal pinna, and commencing action in its pinnules ; action in the primary pulvinus ; action in the other proximal pinna ; long pause ; action in the primary pulvinus of the leaf next below ; long pause ; partial action in its secondary pulvini and pinnules. |
| 26 | Spreading action in the pinnules of that pinna ; long pause ; action in the secondary pulvinus of that pinna, and immediate action in the primary pulvinus ; no further result. |
| 27 | Spreading action in the pinnules of that pinna ; strong action in its secondary pulvinus ; action in the secondary pulvinus of the distal pinna of that side of the leaf ; spreading action in its pinnules ; action in the secondary pulvinus of the other distal pinna ; commencing action in its pinnules, and action in the primary pulvinus ; pause ; action in the other proximal pinnæ. |
| 28 | Spreading action in the pinnules of that pinna ; action in all the secondary pulvini and in the primary one ; spreading action in the pinnules of the other pinnæ. |
| 29 | Spreading action in the pinnules of that pinna ; long pause ; action in all the secondary pulvini and in the primary one ; spreading action in the pinnules of the remaining pinnæ. |

TABLE VI.—*General Table of Results of Application of Heat to the tips of Distal Pinnules*—concluded.

| No. of Experiment. | Result. |
| --- | --- |
| 30 | Results as in No. 28. |
| 31 | Spreading action in the pinnules of that pinna; pause; action in the secondary pulvinus of the affected pinna and in that of the distal pinna of the same side; commencing action in the pinnules of the latter pinna, and action in the primary pulvinus; action in the other pinnæ. |
| 32 | Spreading action in the pinnules of that pinna; action in its secondary pulvinus and in that of the distal pinna of the same side of the leaf; commencing action in the pinnules of that distal pinna, and action in the primary pulvinus; pause; action in the remaining pinnules of the first distal pinna, and action in the other pinnæ; action in one leaf next above. |
| 33 | Spreading action in that pinna; action in the remaining pinnæ successively from the injured side of the leaf to the other; no action in the primary pulvinus. |
| 34 | Spreading action in that pinna; action in all the secondary pulvini and in the primary one; action in the pinnules of the remaining pinnæ. |
| 35 | Slow and irregular action in that pinna; no further result. |
| 36 | Spreading action in that pinna; pause; action in that secondary pulvinus; action in the primary pulvinus, and commencing action in the distal pinna of that side; pause; action in the other pinnæ. |
| 37 | Spreading action in the pinnules; action in the secondary pulvinus of that pinna and in the primary pulvinus; pause; action in the remaining pinnæ. |
| 38 | Slow, spreading action in that pinna; action in its secondary pulvinus; action in the primary pulvinus; pause; slow, centrifugal action in the remaining pinnæ. |
| 39 | Results similar to those in No. 38 save that the secondary pulvinus acted before the basal pinnules. |
| 40 | Results similar to those in No. 38 save that the basal pinnules of the distal pinna of the same side of the leaf had begun to act before the primary pulvinus did so. |

A total of 40 experiments, in only 6 of which was there any extension of movement beyond the primarily affected leaf.

TABLE VII.—*Showing Results of the Application of Heat to the tips of Distal Pinnules when the Plants are dry.*

| No. of Experiment. | Result. |
|---|---|
| | *All the experiments were conducted between 8 and 9 o'clock A.M. of the 8th April. The previous night had been a still cloudy one and little dew had fallen. The morning was still and cloudy, and the sun had not as yet emerged. The brake of Mimosa was perfectly dry, but the leaves in a state of fullest expansion No rain had fallen for more than a week previous to the date of experiment.* |
| 1 | Complete action in that leaf; very long pause; complete action in the first leaf above; no further result. |
| 2 | Action in the affected pinna; convergence of the secondary pulvini; action throughout the other pinnæ; action in the primary pulvinus; pause; action in one leaf above. |
| 3 | Commencing action in the affected pinna; action in the primary pulvinus; complete action throughout the rest of the leaf; no further result. |
| 4 | Complete action in that leaf, the primary pulvinus acting immediately after the affected pinna; long pause; action of the primary pulvinus of the leaf next below; no further result. |
| 5 | Complete action in the affected leaf; long pause; complete action in the first leaf above and the second leaf below; long pause; complete but very slow action in the second and terminal leaf above; no further result. |
| 6 | Action throughout the affected leaf, but depression mechanically obstructed, and pinnular action, in all save the primarily affected pinna, incomplete; long pause; action in the primary pulvinus of the second leaf above; pause; action in one of its secondary pulvini; partial action throughout the greater part of the pinnæ; long pause; action in the pinnules of one of the distal pinnæ of the first leaf above the primarily affected one; pause; action in its primary pulvinus and remaining pinnæ; no further result. |
| 7 | Slow, almost complete action in the affected leaf; no further result. |
| 8 | Slow, almost complete action in the affected leaf; long pause; action in the first leaf above, commencing in the pinnæ and ultimately complete; very long pause; complete action in the second leaf above, commencing in the primary pulvinus; no further result. |
| 9 | Complete action in the affected leaf; action of the primary pulvinus, and incomplete action of the pinnæ of the first leaf above; action in the primary pulvinus, and incomplete action of the pinnæ of the first leaf below; very long pause; incomplete action in the second and terminal leat above; no further result. |
| 10 | Complete action in the affected leaf; no further result. |

TABLE VIII.—*Results of one morning's experiments on pure Mechanical Stimulation of Mimosa pudica.*

| No. of Experiment. | Result. |
| --- | --- |
| | *A.—Depression of the leaf caused by action of the primary pulvinus following pressure on the upper surface of the petiole.* |
| 1 | Full depression ; no further result. |
| 2 | Ditto ditto. |
| 3 | Ditto ditto. |
| 4 | Ditto ditto. |
| 5 | Ditto ; slight action in the secondary pulvin . |
| 6 | Ditto ; no further result. |
| 7 | Ditto ditto. |
| 8 | Ditto ditto. |
| 9 | Ditto ditto. |
| 10 | Ditto ditto. |
| | *B.—Depression of the leaf caused by touching the inferior surface of the primary pulvinus.* |
| 11 | Full depression ; no further result. |
| 12 | Ditto ditto. |
| 13 | Ditto ; slight action in the secondary pulvini. |
| 14 | Ditto ditto ditto. |
| 15 | Ditto ; no further result. |
| 16 | Ditto ditto. |
| 17 | Ditto ditto. |
| 18 | Ditto ditto. |
| 19 | Ditto ditto. |
| 20 | Ditto ditto. |

TABLE VIII.—*Results of one morning's experiments on pure Mechanical Stimulation of Mimosa pudica*—continued.

| No. of Experiment. | Result. |
|---|---|
| | *C.—Action in individual pinnules due to elevation or depression of their tips or touching the upper surfaces of their bases.* |
| 21 | Action in that pinnule ; no further result. |
| 22 | Ditto ditto. |
| 23 | Ditto ditto. |
| 24 | Ditto ditto. |
| 25 | Ditto ditto. |
| 26 | Ditto ditto. |
| 27 | Ditto ditto. |
| 28 | Ditto ditto. |
| 29 | Ditto ditto. |
| 30 | Action in that and the opposite pinnule. |

TABLE IX.—*Results attending Mechanical Obstruction to the depression of the Petiole on action of the Primary Pulvinus. All the experiments carried out on one morning by section of distal pinnules.*

| No. of Experiment. | Result. |
|---|---|
| | *A.—Depression of the leaf due to action of the primary pulvinus mechanically obstructed.* |
| 1 | Tips of distal pinnules cut off. The cut pinnules acted at once ; pause ; action in primary pulvinus ; pause ; very imperfect centripetal action in the cut pinna ; no further result. |
| 2 | Procedure and results as in No. 1. |

TABLE IX.—*Results attending Mechanical Obstruction to the depression of the Petiole on action of the Primary Pulvinus*—continued.

| No. of Experiment. | Result. |
|---|---|
| 3 | A leaf with two large pinnæ. Action in cut pinnules; pause; action in primary pulvinus; very slow, partial action in 8 pairs of pinnules beneath the cut pair; no further result. |
| 4 | Action in the cut pinnules; pause; action in primary pulvinus; centripetal action in the distal and basal portions of the cut pinna; slight action in the central portion; no further result. |
| 5 | Action in the cut pinnules; action in the primary pulvinus; partial, centripetal action in the distal and basal portions of the cut pinna; no action in the central portion; no further result. |
| 6 | Slow, spreading centripetal action in the outer part of the cut pinna; action in the primary pulvinus; very slow and partial; centripetal action in the basal part of the cut pinna; no further result. |
| 7 | Action in the cut pinnules; action in the primary pulvinus; partial action in the distal and basal thirds of the cut pinna; complete action in the central third; no further result. |
| 8 | Almost complete centripetal action in the cut pinna; action in the primary pulvinus; no further result. |
| 9 | Action in the cut pinnules; pause; action in the primary pulvinus; almost complete, slow, centripetal action in the cut pinna; no further result. |
| 10 | Slow, progressive, complete centripetal action in the cut pinna; long pause; action in the primary pulvinus; partial, irregular, centrifugal action in another pinna; very long pause; slight, slow action in the basal part of another pinna; no further result. |
| 11 | Action in the cut pinna; action in the primary pulvinus; pause; action in one other pinna; no further result. |
| 12 | Action in the cut pinnules; pause; action in the primary pulvinus; partial and incomplete centripetal action in the cut pinna; action in the basal half of one other pinna; no further result. |
| | *B.—Depression of the leaf due to the action of the primary pulvinus fully carried out.* |
| 13 | A two-pinnaed leaf. Pause; spreading, centripetal action in the cut pinna; full depression; well pronounced action in the basal half of the other pinna. |
| 14 | Slow, almost complete action in that pinna; pause; full depression; no further result. |
| 15 | Action in the cut pinnules; slow, progressive, centripetal action in that pinna; full depression; almost complete, spreading, centrifugal action throughout the other pinnæ. |

TABLE IX.—*Results attending Mechanical Obstruction to the depression of the Petiole on action of the Primary Pulvinus*—concluded.

| No. of Experiment. | Result. |
|---|---|
| 16 | Slow, progressive, almost complete action in that pinna ; full depression ; action in basal half of the next pinna ; no further result. |
| 17 | Slow, progressive action in that pinna ; full depression ; no further result. |
| 18 | Slow, progressive action in that pinna ; pause ; extreme depression ; gradual, complete centrifugal action in the other pinnæ. |
| 19 | Slow, progressive action in that pinna ; pause ; full depression ; almost complete, centrifugal action in the basal two-thirds of the other pinna. |
| 20 | Slow progressive, complete centripetal action in that pinna ; pause ; full depression ; pause ; slow, centrifugal action in basal halves of two other pinnæ. |
| 21 | Slow, progressive, complete action in that pinna ; pause ; extreme depression slow, centrifugal action in the basal two-thirds of the other pinnæ. |
| 22 | Ditto ditto ditto. |
| 23 | Slow, progressive, centripetal action in that pinna ; pause ; full depression ; action in basal halves of the other pinnæ. |
| 24 | Ditto ditto ditto. |

In the above table we have the results of twenty-four experiments conducted on the same morning on leaves under precisely similar circumstances save that in twelve of the cases depression of the petiole was mechanically obstructed or prevented, whereas in the remaining twelve it was fully carried out. In three of the first set action was manifested in other pinnæ than the cut one ; in ten of the second set a similar phenomenon was present.

TABLE X.—*Results of Application of Heat to the axes of Mimosa pudica.*

| No. of Experiment. | Result. |
|---|---|
| 1 | *Heating effected by means of coiling one extremity of a piece of copper wire round the axes and applying a spirit-lamp flame to the free extremity.*—Centrifugal action of all the leaves above the point of application ; no action below. |

TABLE X.—*Results of Application of Heat to the axes of Mimosa pudica* —continued.

| No. of Experiment. | Result. |
| --- | --- |
| | *Heating effected by the application of the tips of heated forceps.* |
| 2 | *Heat applied to the axes close beyond a proximal leaf and far from the next distal one.*—Progressive centrifugal action along the whole course of axis. No action below point of application. |
| 3 | *Ditto* ditto ditto. |
| 4 | *Ditto* ditto ditto. |
| 5 | *Ditto* ditto ditto. |
| 6 | *Ditto* ditto ditto. |
| 7 | *Ditto* ditto ditto. |
| 8 | *Procedure as in the previous cases.*—Results as in the previous cases save that the primary pulvinus of the first leaf beneath the point of application acted. |
| 9 | *Ditto* ditto ditto. |
| 10 | *Forceps applied nearer inferior than superior leaf.*—Progressive complete action of all the leaves above; no action below. |
| 11 | *Application as in No. 10.*—Action in all the leaves above; no action below. |
| 12 | *Ditto* ditto ditto. |
| 13 | *An old woody axis with three leaves with rigid primary pulvini above the point of application of the forceps, which was immediately beyond the first inferior leaf.*—Action in the secondary pulvini and pinnules of the first leaf above; no action below. |
| 14 | *Procedure as in No. 10.*—Results as in No. 10. |
| 15 | *Ditto* ditto. |
| 16 | *Forceps applied very close to the inferior leaf.*—Action in the inferior leaf and in all the distal ones. |
| 17 | *Procedure as in No. 15.*—Results as in No. 15. |
| 18 | *Procedure as in No. 17.*—Action in the first leaf above occurred 17 seconds after application. |

TABLE X.—*Results of Application of Heat to the axes of Mimosa pudica* —continued.

| No. of Experiment. | Result. |
|---|---|
| 19 | *Procedure as in No. 17.*—First leaf above acted after an interval of 20 seconds; there was then a pause of one minute, followed by action in the second and third leaves above; no action in the leaf beneath, although the point of application was very close to it. |
| 20 | *Forceps applied very close to inferior leaf.*—Almost immediate action in the primary pulvinus alone of the inferior leaf; slow, progressive, complete action in all the leaves above. |
| 21 | *Application very close to the inferior leaf.*—Slow, centrifugal action in all leaves above; no action below. |
| 22 | *Ditto* ditto ditto. |
| 23 | *Ditto* ditto ditto. |
| 24 | *Ditto* ditto ditto. |
| 25 | *Application very close to base of inferior leaf.*—Very slow, protracted, centrifugal action in all the leaves above; the primary pulvini acted at long intervals, and the pinnæ and pinnules remained for long expanded; no action below. |
| 26 | Slow, progressive, centrifugal action in all leaves above; no action below. |
| 27 | *Application far from any superior leaf.—Experiment just after heavy rain.* Spreading, centrifugal action in the primary pulvini of all leaves above; slight action in secondary pulvini and pinnules; no action below. |
| 28 | *Application very close to base of inferior leaf.*—Spreading, centrifugal action in all the leaves above; action in primary pulvinus, and slight action in pinnæ of leaf next below. |
| 29 | *Application as in No. 28.*—Action in the leaf next above; no further result. |
| 30 | *Application as in No. 29.*—Action in the pinnæ of the two next leaves above; no action in their primary pulvini or elsewhere. |
| 31 | *The two leaves next above the site of application had each a young axillary leaf.*—Action in these young leaves; no further result. |
| 32 | *Application as in No. 29.*—Action in axillary leaf of first old leaf above; long pause; action in axillary leaf of next superior leaf; long pause; action in axillary leaf of first inferior leaf; pause; action in primary pulvinus of first old leaf above the site of application. |

TABLE X.—*Results of Application of Heat to the axes of Mimosa pudica* —continued.

| No. of Experiment. | Result. |
| --- | --- |
| 33 | *Application as in No. 29, the points of the forceps almost touching the pulvinus of the leaf next below.*—Slow action in first two leaves above; no action below. |
| 34 | *Application as in No. 29.*—Very long pause; action in the primary pulvinus of leaf next above; very long pause; commencing action in the pinnæ of the first leaf, and action in primary pulvinus of the next leaf above; pause; action in the pinna of the second leaf; no action below. |
| 35 | *Application as in No. 29.*—Long pause; action of leaf next above; very long pause; action in terminal leaf of axis separated by six internodes from the previous leaf; action in the leaf below the terminal one; pause; action in leaf above that one which acted first; no further action. |
| 36 | *Application as in No. 29.*—Pause; action in leaf next above; short pause; action in leaf next below; very long pause; action in primary pulvinus of second leaf above site of application; long pause; action in its pinnæ; no further result. |
| 37 | *Forceps very strongly heated.*—Almost immediate action in leaf next below; long pause; action in primary pulvinus of first leaf above; pause; progressive, centrifugal action in its pinnæ; very long pause; action of the third leaf above the site of application; partial and slow action in pinnæ of the second leaf above the site of application; no further result. |
| 38 | *Application as in No. 29.*—Action in leaf next above; action in leaf next below; action of the second leaf beneath that (the intermediate leaf was at a rooted node); action in the first leaf of the axillary shoot of the leaf above the point of application; no action in the second leaf above, which was situated at a rooted node. |
| 39 | *Application as in No. 29.*—Action in all the leaves above the point of application; no action below. |
| 40 | *Application as in No. 29.*—Action in fourteen leaves on axis and shoots above, the primary pulvini alone acting in the more distal ones; no action below. |
| 41 | *Application immediately above the inferior leaf.*—Slow, progressive, centrifugal action in the primary pulvini of all the leaves above; the secondary pulvini and pinnules only acted in some cases, and in these after long intervals; no action below. |
| 42 | *In this experiment the flame of a wax match was applied direct to the axis.*—Action in four leaves above; no action in the fifth and terminal one; no action below. |

TABLE X.—*Results of Application of Heat to the axes of Mimosa pudica* —continued.

| No. of Experiment. | Result. |
|---|---|
| 43 | *Sun's rays focussed by a lens on the axis close to the inferior leaf and about two inches from the one next above.*—Spreading, centrifugal action in all the leaves above; no action below. |
| 44 | *Application as in No. 43, the nearest distal leaf being about 3 inches from the site of application.*—Action in all distal leaves; no action below. In both 43 and 44 the first inferior leaf responded readily to direct stimulation, and in both the effect on the axis at the site of application was to cause exudation of a drop of fluid, but no visible discolouration. |
| 45 | *Application as in No. 44.*—Exudation of fluid; action in all leaves above; no action below. |
| 46 | *Application as in No. 45.*—Exudation of a drop of fluid; action in all leaves above; action in two leaves below. |
| 47 | *Application as in No. 46, close to inferior leaf and 1·5 inches from the superior one.*—No exudation; action in four leaves above; no action below. |
| 48 | *Application as in No. 47.*—Slight discolouration; no exudation; action in all the distal leaves; action in the leaf next below; no further action. |
| 49 | *Application as in No. 48.*—Action in three leaves above; no action below. |
| 50 | *Application as in No. 49.*—Visible exudation of a small drop of fluid; action in one leaf above; no action below. |
| 51 | *Application as in the previous cases, but about midway between the superior and inferior leaves.*—Slight discolouration; no visible exudation; action in all nine distal leaves; no action below, although all the leaves readily responsive to direct stimulation. |
| 52 | *Application as above, but close to the inferior leaf and about 1·5 inches from the superior one.*—No visible discolouration or exudation; action in all distal leaves; action in one leaf below. |
| 53 | *Application as above.*—Discolouration and escape of fluid; action in four leaves above; no action below. |
| 54 | *Application as above.*—Discolouration and escape of a large drop of fluid; centrifugal action in six leaves above; no action below. |
| 55 | *Application as above, close to the inferior leaf.*—Discolouration, but no visible exudation; action in four distal leaves; slow action in first inferior leaf. |

TABLE X.—*Results of Application of Heat to the axes of Mimosa pudica* —continued.

| No. of Experiment. | Result. |
|---|---|
| 56 | *Application as above, midway between inferior and superior leaves.*—Slight discolouration; action in four distal leaves; no action below. |
| 57 | *Application as above, close to the inferior leaf and about 1·5 inches from the next superior one.*—No exudation; successive centrifugal action in all eight distal leaves; no action below, although the inferior leaf was readily responsive. |
| 58 | *Application as in No. 57.*—No exudation; action in all seven distal leaves; no action below; inferior leaf readily responsive. |
| 59 | *Application as in No. 58.*—Centrifugal action of all distal leaves; action in two leaves below; discolouration; no visible exudation. |
| 60 | *Application as in No. 59, close to the inferior leaf and about 1 inch from the superior one.*—No discolouration; no exudation; action in four leaves above, consisting of partial action of the primary pulvini, and slight action of pinnules; no action below; inferior leaf readily responsive to direct influences. |
| 61 | *Application as in previous experiments near the inferior and distant from the superior leaf.*—Action in the inferior leaf, and spreading centrifugal action in all the distal leaves; discolouration, and visible exudation of a large drop of fluid. |
| 62 | *Application as in No. 61, close to the inferior leaf and about 1·5 inches from the superior one.*—Discolouration; no exudation; progressive, slow, centrifugal action in all distal leaves; no action below. |
| 63 | *Application in No. 62, about 0·75 inches from leaf next below and 4 inches from leaf next above.*—Discolouration, and slight exudation; centrifugal action in all eleven distal leaves; no action below. |
| 64 | *Application as in No. 63, about 0·5 inches from the inferior and 1·75 inches from the superior leaf.*—Slight discolouration; no visible exudation; action in three first leaves above; no action below. |
| 65 | *Application as in No. 64, on a strong, hard axis, close to an inferior leaf and far from the distal one.*—No visible discolouration or escape of fluid; action in several leaves above; no action below. |
| 66 | *Application as in No. 65, about 0·5 inches from next leaf below and 13 inches from leaf next above.*—At one and a half minutes from the cessation of the application the leaf below and the two first leaves above acted; at two minutes the third leaf above the site of application acted; at 2·25 minutes the fourth and terminal leaf acted. The axis was a hard and dry one, and the application caused discolouration, but no exudation. |

TABLE X.—*Results of Application of Heat to the axes of Mimosa pudica* —concluded.

| No. of Experiment. | Result. |
| --- | --- |
| 67 | *Application as above, about 0·25 inches from leaf next below and 3 inches from leaf next above.*—The axis a strong, woody one ; slow, progressive, centrifugal action in all distal leaves ; long pause ; action in the leaf next below ; no further action. |
| 68 | *Application as above.*—Discolouration and slight exudation ; action in all eight distal leaves ; action in first leaf below when centrifugal action had almost reached the extremity of the axis ; no further action. |

A total of 68 experiments. In all of them centrifugal, propagation occurred, and in 50 centrifugal propagation only was present. In the 18 cases where phenomena of centripetal propagation was present, it was very limited in amount, in no cases advancing beyond the two nearest leaves. Of the total 68 experiments, 41 were carried out in the period from August to December, and 27 from December to March, and each of these groups furnished 9 cases of centripetal propagation. The phenomenon of centripetal propagation would from this seem to occur more frequently during dry than moist periods.

TABLE XI.—*Results of section of Tips of Axes in Mimosa pudica.*

| No. of Experiment | Result. |
| --- | --- |
| | The tips of axes gently cut off by means of a sharp scalpel or pair of scissors. |
| 1 | Action in first four leaves beneath the tip · no action in the next three leaves action in the five following leaves. |
| 2 | Exudation of a large drop of fluid ; long pause ; action in leaf next below. |
| 3 | Exudation ; long pause, action in two leaves next below. |
| 4 | Action gradually propagated centripetally along the axis as far as it was traceable. |

TABLE XI.—*Results of section of Tips of Axes in Mimosa pudica* —continued.

| No. of Experiment. | Result. |
|---|---|
| 5 | Results as in the previous experiment. |
| 6 | No result. The axis was a dry, hard one. |
| 7 | Progressive, centrifugal action in nine leaves below. |
| 8 | A long pause ; action in first leaf below. |
| 9 | Progressive, gradual, centripetal action in ten leaves below, *i.e.*, along entire course of axis traceable. |
| 10 | Action in first leaf below ; long pause ; irregular action along entire course of axis traceable ; some leaves acting, others situated at various points failing to act. |
| 11 | Very long pause ; action in first leaf below. |
| 12 | Progressive action of five leaves situated at intervals along the axis. |
| 13 | Very long pause ; action in the first leaf below. |
| 14 | Long pause ; action in the first leaf below. |
| 15 | Successive action in the three upper leaves. |
| 16 | Irregular action along the axis as far as traceable. |
| 17 | Regular centripetal action along all of the axis traceable, including nine leaves. |
| 18 | Irregular action along all of the axis traceable. |
| 20 | Ditto ditto. |
| 21 | Very long pause ; action in first inferior leaf |
| 22 | Very long pause ; irregular, centripetal action along the axis. |
| 23 | Long pause ; action of superior leaf. |
| 24 | Ditto ditto. |
| 25 | Long pause ; regular centripetal action along the axis. |
| 26 | Pause, irregular, centripetal action along the axis. |

TABLE XI.—*Results of section of Tips of Axes in Mimosa pudica* —continued.

| No. of Experiment. | Result. |
|---|---|
| 27 | Regular, centripetal action along the axis so far as traceable. |
| 28 | Exudation of a large drop of fluid; pause; action in the third, fourth, fifth, and sixth leaves from the tip; no action in the first and second; no action in the seventh; action in the eighth; no action in the ninth; action in the tenth. |
| 29 | Exudation; pause; action in the second, third, fourth, fifth, and sixth leaves below; action in the ninth and tenth leaves; action in the first leaf; no action in the seventh and eighth leaves. |
| 30 | Exudation; pause; action in the second, third, fourth, fifth, sixth, and seventh leaves below; action in the first leaf; action in other leaves successively along the axis so far as traceable. |
| 31 | Action in fourth, fifth, and sixth leaves below. |
| 32 | Exudation of a very large drop; pause; centripetal action of all leaves successively along the course of the axis so far as traceable. |
| 33 | Exudation of a large drop; successive centripetal action in the primary pulvini only of eight leaves below. |
| 35 | Exudation; pause; action in third, fourth, fifth, and sixth leaves below; action in first leaf below; action of several leaves successively at wide intervals along the axis. The primary pulvini alone acted in some leaves, whilst in others complete action took place. |
| 36 | Exudation; pause; action in third, fourth, fifth, seventh, eighth, and ninth leaves; action in first leaf; long pause; action in second leaf. The primary pulvinus alone acted in most of the leaves. |
| 37 | Exudation; action of first, second, third, fourth, sixth, eighth, and ninth leaves. The primary pulvinus alone acted in most cases. |
| 38 | Action in fourth leaf below; action in third leaf; progressive centripetal action along the course of the axis; long pause; action of first leaf. |

A total of 38 cases, demonstrating the possibility of very extensive centripetal propagation under certain conditions, and also showing the frequence with which the phenomena of movement take place irregularly along the axis.

TABLE XII.—*Showing Results of section of Distal Pinnules in young heavy Leaves.*

| No. of Experiment. | Result. |
| --- | --- |
| 1 | Action in the cut pinnules; pause; action in the primary pulvinus; pause; action in the pinnules of that pinna; no further result. |
| 2 | Action in the cut pinnules; pause; violent and extreme action in the primary pulvinus; pause; centripetal action in one or two distal pinnules of the cut pinna; no further result. |
| | *The other pinnules responded readily to mechanical impulses.* |
| 3 | Action in the cut pinnules; pause; action in the primary pulvinus; spreading, centripetal action in the cut pinna; pause; nearly complete; action in the other distal pinna; no further result. |
| 4 | Action in the cut pinnules; pause; progressive action in the cut pinna; pause; action in the remaining pinnæ; action in the primary pulvinus. |
| 5 | Action in that pinna only. |
| 6 | Pause; action in the primary pulvinus; action in the distal two-thirds of the cut pinna; pause; action in the basal third; no further result. |
| 7 | Pause; action in the primary pulvinus; action in the pinnules of that pinna; no further result. |
| 8 | Results as in No. 7. |
| 9 | Ditto. |
| 10 | Ditto. |

This table illustrates two points very clearly—the discontinuity of action under certain conditions, and the limitation of action under the influence of others. All these experiments were conducted between 7 and 8 o'clock of a warm, moist, still morning, on leaves which had not yet been exposed to direct sunshine and which were loaded with moisture. They were all large, young leaves, and in the majority of cases the weight of the pinnæ obviously strained on the primary pulvinus. Under these conditions there was a special reason why any loss of fluid should tell with special effect on the primary pulvinus, and at the same time a reason for only limited loss of fluid. The result ought to be specially rapid action in the primary pulvini and only limited action elsewhere, and such results were actually present, as the table shows.

Action in the primary pulvinus preceding centripetal action in the cut pinna appears to be the normal phenomenon under certain conditions. It may be looked for as almost certain to occur in young, heavy leaves, in which, from the position and direction of the petiole, a special strain is evidently exerted on the primary pulvinus in maintaining the erect position. In old heavy leaves, specially if loaded with dew or rain, similar phenomena frequently manifest themselves. They do not, however, occur with such constancy as in the case of the young leaves. This is to be credited to the alteration in the character of the pulvinar tissues accompanying increased age. In the leaves of mimosa pudica the tissue of the under-half of the primary pulvinus is relatively weakest when the leaves arey oung and becomes gradually stronger and more resistant with increasing age. So much does this take place in certain cases that it is not uncommon to meet with old but otherwise healthy leaves in which the primary pulvinus has become rigid and in which accordingly petiolar depression is impossible. This increase in strength in the normally weakest tissue with increasing age is not peculiar to the pulvini of *Mimosa pudica*, much more conspicuous examples of it are present in other plants. For example, in *Bauhinias* and in *Albizzia Lebbek* the young leaves exhibit extreme nyctitropic movements which gradually diminish and ultimately absolutely disappear with increasing age and thickening and rigidity of the primarily passively weaker portions of the pulvinar tissues.

TABLE XIII.—*Results of focussing the Sun's rays on the upper surfaces of the primary Pulvini of Mimosa pudica.*

| No. of Experiment. | Result. |
|---|---|
| 1 | Depression of the petiole. |
| 2 | Extreme elevation of the petiole, followed by depression. |
| 3 | Depression of the petiole. |
| 4 | Rotation of the petiole. |
| 5 | Depression of the petiole. |
| 6 | Strong elevation, followed by depression. |
| 7 | Strong direct elevation, followed by strong direct depression. |
| 8 | Elevation of the petiole. |

TABLE XIII.—*Results of focussing the Sun's rays on the upper surfaces of the primary Pulvini of Mimosa pudica*—continued.

| No. of Experiment. | Result. |
|---|---|
| 9 | Depression of the petiole. |
| 10 | Ditto. |
| 11 | Strong elevation; a pause; action in the pinnæ; nothing more. |
| 12 | Depression of the petiole. |
| 13 | Strong elevation, followed by action in the pinnæ, and depression of the petiole. |
| 14 | Elevation; a pause; action in the pinnæ; nothing more. |
| 15 | Elevation; a pause; depression. |

A total of 15 experiments tried on two successive mornings and in which phenomena of elevation manifested themselves in nine instances.

# Note on some Aspects and Relations of the Blood-organisms in Ague.

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Respecting abnormal aspects of the blood in fevers termed "malarial," no fresh information had been acquired of late; nor had the bacillary structures, described to me at Rome in 1880-81 by Professors T. Crudeli and Marchiafava, been witnessed subsequently at Bombay; and hence this subject (others intervening) had remained in abeyance until the present year, when the striking confirmation of Dr. Laveran's researches by Professor Osler, published in the *British Medical Journal* of 12th March 1887, compelled, as it were, renewed attention. In July last, I therefore regularly examined the blood of a man affected with quartan ague (case No. 1 below), and detecting in it at fever-periods many pigmented spherules, I next found (in case No. 2) the equally characteristic crescentic bodies; after which further enquiry was continued until October, with the results now noted.[1]

It has thus been already ascertained that in Western India the so-termed "hæmatozoa of malaria," are readily to be discerned; and, as this datum will doubtless apply to India generally, the conclusions arrived at here, as well as elsewhere, can be tested without delay.

Dr. A. Laveran's original work, the "*Traité des Fièvres palustres*, Paris, 1884," contains a full account of his "microbe du paludisme" in its ordinary free forms, all of which I have seen; and, subsequently, to those Algerian discoveries of 1880-81, Dr. Marchiafava and several observers in Italy have added to the list of "malaria-organisms" other minuter forms sessile within the red blood-discs, which Dr. Osler in America also fully recognises, and which to a certain extent have been verified here.

While so far in accord, the results newly acquired at Bombay yet differ in some respects, and will therefore be given in detail: the kind and source of data being first stated, next the organisms described, and lastly some of their relationships discussed.

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[1] During November some further data were acquired, which are alluded to in a Note at the end.

1.—The Data.—Material used was derived from native hospital-patients, the fresh blood being taken from a cleansed finger by means of a bright needle, in quantity a quarter-drop, so thinly spread that the red-discs may mostly be disengaged and lie flat in the field, with only the gentlest pressure and as free as practicable from foreign admixture. First moistening with the breath cover-glass and slide, will favour the required dispersion of the red corpuscles, and has no apparent distorting effect. The temperature of the air was uniformly at 75° to 80° F., and its humidity considerable at the rainy season prevailing. The $\frac{1}{10}$ inch water-immersion, or $\frac{1}{12}$ inch oil-immersion, lenses of London make, with achromatic condenser, were, by preference, commonly employed; the dry $\frac{1}{8}$ inch objective lens being suitable only for preliminary search. Bright sunlight is almost indispensable, since the best lamp-light proved unsatisfactory; and examination of the blood must be promptly made, in order to detect any active flagellate movements of organisms present. When long watching is intended, the edges of the specimen may be sealed. Permanent preparations, made by drying over the fumes of osmic acid, proved hardly satisfactory; but the ordinary staining processes serve well for crescents and bare spherules; flagellate appendages being, however, only occasionally thus preserved.

The cases investigated were taken indiscriminately as admitted, and before the administration of quinine. Commonly one careful scrutiny was made of each, preferably at time of febrile exacerbation, and always repeated when any doubts arose; and since in this way the organisms present were always promptly detected, when appearances were negative I inferred their absence, at least in the free state; not relying so much on minute changes within red-discs, or on slighter pigmentation of leucocytes. As patients presented themselves at various periods of illness, it may be supposed that most confirmed or later stages of "fever" came under notice; and in three of the positive instances narrated, the actual initiation of fresh pyrexia was repeatedly witnessed in hospital. In the list below the usual names of diseases have been retained, as was needful; it being well-known, however, that they may be somewhat vaguely applied.

Series A, *Malarious Fever*—73 instances. Intermittents 18 (of which 6*); remittents—simple 22 (of which 2*), complicated 14,—total 36; splenic cachexia 19 (of which 1*, non-febrile).

Series B, *Non-malarious Fevers*—27 instances. Simple continued fever 4; enteric 1; fever symptomatic of bronchitis, pneumonia, pleurisy phthisis, hepatitis, dysentery, pharyngitis, cerebral lesion, 22: grand total 100.

The instances marked by the asterisk* were those with pigmented organisms, either free or both sessile and free; amounting to 9 per cent. of the whole, or of series A, to which they were solely confined, to about 13 per cent. Respecting this malarial group, I consider it open to personal judgment whether or not the presence of even a very few pigmented red blood-discs and white cells,

should entail a positive diagnosis of actual malarial infection; and were the affirmative strictly maintained, I doubt not some of the instances above would be differently estimated, and the asterisk numbers be correspondingly increased. This qualification (and it may be there are others) it is right to mention here; and the remark applies also to series B, wherein some cases affected with a "malarious taint" (so called) must on current views have been likewise included.

Adverting now to the positive instances, I note that in the 9 examples 2 of intermittents showed pigmented spheroids so few and seldom that it will suffice to mention the fact; both, clinically, being of mild and irregular illness. The remaining 7 were under continuous daily observation from three to six weeks, or more, and were closely watched: all being adult males, as regards history, aspect, and severity of illness none were obviously peculiar (only that quartan ague is seldom seen at Bombay); none were fatal, or complicated with marked local inflammation; and, briefly, their ascertained blood-state alone served as a distinctive feature. None were first attacks, and none were seen at early stage of illness. The intensity, form, and duration of fever were varied; and once no actual pyrexia was ever present. The general condition of patients was fair or even good, and but twice seriously impaired; the splenic enlargement being never extreme, and uneasiness always either moderate or slight. The quartan ague of No. 1 was typical, and distinct quotidian and tertian paroxysms were watched in Nos. 3 and 4. Treatment by quinine prior to admission was usually denied, and subsequently this drug was purposely withheld so long as deemed desirable. If these instances be few in number, still they are all genuine and unselected representatives of ordinary experience; and the study of each being more sustained than in records I have met with, the results attained seem as trustworthy as could be expected in so plain a research. The notes following are much condensed, and they are arranged so as to permit of independent judgment and future comparison. These seven patients were 203 days in hospital (of which 40 fever and 163 non-fever days); and their blood was examined, commonly twice and frequently oftener, on 127 days (of which 29 fever and 98 non-fever days); blood organisms being found on 109 days, and not seen on 18 days of later convalescence after fever.

CASE 1.—*Quartan ague.*—I. P. N., Goanese, æt. 28, servant on board an ocean-going steamer, had never suffered from fever until three months ago, when seven days after landing for a day at Colombo, he was seized while *en route* to Bombay. Apparently the type of fever contracted was that of an obstinate intermittent. After his admission here in August, the symptoms were those of regular quartan ague, seven paroxysms being witnessed: upon the exhibition of arsenic only a single interruption ensued with no other benefit obvious, and the attacks again augmenting (last t. 105°F.), two 10-grain doses of quinine were administered, with the effect of perfectly arresting all further manifestation of fever after one quasi-abortive periodic rise: the doses of quinine being reduced

to 5 grains for eight days, the man begged discharge (cured) twenty days after the last febrile paroxysm, and 40 after admission. At first, the area of splenic dulness measured about 5 square inches, finally becoming nearly normal.

The blood was examined on twenty-six days, *viz.*, about dates of every relapse, also during the apyretic intervals, and often subsequent to the latest paroxysm seen. At febrile periods, pigmented spheroids, both sessile and free, became numerous with the onset of fever, the free organisms breaking up and dispersing during and after the acme of pyrexia; and at apyretic intervals, only some pigmented leucocytes might be seen in the blood. At no time, in this case, were the crescentic bodies found; but on the third day, after the last paroxysm of fever (quinine previously given), I detected active, flagellated spherules in the blood (t. 98°) at 9 A.M; at 1-45 P.M., only a few bare spheroids, so at 2-15 P.M., and again at 6 P.M., as well as on the following morning; after which date, for fourteen days longer, the blood remained free from visible contamination, except as indicated by scanty brown and blue pigment granules free. It was noted that at change of date of paroxysm under the influence of arsenic, a few spherules appeared in the blood about the time of expected febrile relapse, yet without the concurrence of any rise in temperature.

2. *Quotidian ague.*—C. A., Frenchman, æt. 26, country-born, an engineer's assistant, contracted ague whilst engaged on reclamation-works at Singapore about a year since (1886), and was obliged to leave: subsequently, in India, he had no return of the fever until June last, whilst on deck-passage (bad weather) to Bombay. Resorting to an hospital, his illness was soon arrested here by quinine, but, again returning, he entered the J. J. Hospital, where in my ward a series of eleven daily paroxysms were watched (max. t. 102-3° F.), until checked by quinine (20 grs.); after which no return for fifteen days (quinine in smaller doses), and also outside hospital for another period of ten days without medicine. The spleen was at first distinctly enlarged, the dulness-area measuring 5 inches by 8 inches—though not markedly tender: it soon after subsided. The man's health improved considerably in hospital.

The blood displayed numerous pigmented crescents, with a few spherules, on his admission and many times subsequently, both during and after the febrile manifestations; the organisms finally becoming fewer, though with care detectible until the last day of his stay in hospital: and, in addition, I detected on the fourth day after arrest of fever (t. 98°) several active flagellate bodies, which were again seen upon several occasions until the end. These organisms were, with the patient, shown at the September meeting of the local medical society and freely recognised by members present. The patient could not be induced to remain longer than a month, and on two subsequent visits he made I did not perceive any organisms in the blood.

3. *Quotidian (? remittent) fever; intercurrent paroxysms.*—A. I., Mussulman, æt. 20, a dock labourer, born and resident in Bombay, had fever seemingly paroxysmal for twelve days, which spontaneously subsided the day after his admission (August), when t. 101·4° and diaphoretics alone given. The spleen was then a little enlarged, but not tender. Quassia was given, and after ten days complete apyrexia a series of smart intermittent paroxysms spontaneously began, the first three being of tertian and the last three of quotidian type, (max. t. 104° to 105·2°): quinine was then administered in three 10-grain doses, and the fever became forthwith arrested; there being no recurrence during the twenty-two days of his further stay in hospital, during which time the cinchona alkaloids alone were exhibited.

The spleen became decidedly again enlarged—dulness-area $3\frac{1}{2}$ inches by 5 inches—with this recurrence of fever, subsiding with its close. Finally, the patient's health seemed fully restored, when he left hospital after a detention of six weeks. Respecting the origin of the malarial infection in this instance, it appeared that the disease might have been acquired outside the town of Bombay, when last year during the rains the patient had lived at Kallian (a malarious locality near) for three months, working as a weaver and then suffering from fever, though not again since his return to Bombay until the present wet season. No prior treatment.

The blood, at subsidence of fever on his admission, showed several large pigmented crescentic bodies, and a few spherules: these persisted during apyrexia until the inter-current febrile attack, when for the whole period of eight days I failed except once to detect any organisms in the blood. Yet after the first dose of quinine, with complete arrest of fever, the crescents and spherules became again apparent and so continued, though diminishing in numbers, until the patient's discharge. It was doubtful if the flagellate spheroids were ever present, but from some appearances of active pigmental movements, I considered it likely that they might have been overlooked on both first and second occasions of defervescence.

4. *Intermittent fever* (*mild*).—I. de S., Goanese, æt. 22, a ship's steward, never subject to fever until landing last month (July) he went to his "*coor,*" or club, at Cavel (a fever locality in Bombay); and then, after a wetting, he suffered from an attack, which subsided under treatment, but had returned a week before his admission. The day after entry, his temperature was normal and remained so for five days; when there began spontaneously a series of mild daily paroxysms (max. t. 102° F.) for four days, and then two quasi-tertian exacerbations (t. 100° F.), after which the patient decamped. The spleen at all times was but slightly enlarged, its dulness-area not exceeding 2 inches by $3\frac{1}{2}$ inches. Quinine was not given; but only quassia and salines.

The blood on the day after admission (t. 98·5°) displayed both crescents and spherules, there being also indications, as I thought, of the flagellate form: later, during apyrexia, the crescentic bodies, and at times of renewed pyrexia both the crescentic and spheroidal, with on the fourth day (t. 100°) distinct flagellar organisms: subsequently, the two more common forms were repeatedly seen.

5. *Malarial cachexia: no pyrexia in hospital.*—B. M., Mussulman, æt. 40, native of Bokhara, resident two years in Bombay, a knife-grinder, was admitted in August with colicky pains, a history of recent fever (? ague) and the spleen enlarged, reaching 1 inch below the costal margin and having a dulness-area of 5 by 8 inches: he had also had fever in his native country. General condition fair, but aspect pallid: the temperature, pulse, and respiration were carefully watched, and at no time was any febrile excitement detected throughout his detention of six weeks' duration. Arsenic was long administered, and once two doses of quinine (each 10 grains), which produced little or no apparent general effect; finally, a sedative soda-mixture was given. His body weight at entry was $110\frac{1}{2}$ lbs. and 110 lbs. at discharge.

About a week after this man's admission, my colleague, Surgeon Manser, detected some crescentic organisms in his blood; and three days later I found, in addition, spheroidal bodies, both bare and flagellate, which during the next nine days were often seen in large numbers. The dose of quinine being now given, crescents and spherules still abounded for twelve days longer at least (arsenic being resumed); but the flagellate organisms were not again seen: soon afterwards, only crescentic bodies could be found after long search, and for a week before the patient's discharge the blood seemed free of contamination.

6. *Continued* (*remittent*) *malarious fever*.—A. R., Mussulman, æt. 24, a railway employé, three years in Bombay, but lately resident for three months at Ahmedabad, whither he had gone for work and where he fell ill: was brought to hospital 13th September by the police from a neighbouring hamlet, where he had been found in a state of great prostration while on his way back to Bombay. Temperature on admission 105°, pulse 124, respirations 24; next morning t. 99·8°, rising to 104·8°, in spite of two 10-grain doses of quinine; after declining on the following day, however, fever did not again recur during the remainder of his month's stay in hospital. At first the spleen was but moderately enlarged, dulness reaching from eighth rib to ½ inch below costal margin, and the organ was not particularly tender: soon afterwards it subsided to normal dimensions The predominant early symptoms were pronounced anæmia and extreme debility, a hæmic bruit and dicrotous pulse; about six days after the cessation of fever an anal abscess formed, brief hemicrania set in, and six days later there was noted some anasarca, most perceptible in the feet, where desquamation of the sole occurred: no hæmorrhagic spots, and no albumen in the urine; convalescence steady and complete. This patient had been liable to fever at his home in the Deccan: the present attack had come on three weeks before admission, fever being at first intermittent and then persistent, the chills very marked and no medicine taken. In hospital quinine was given daily (22 grains) for ten days, and followed by cinchona alkaloids and iron.

The blood was examined during the febrile remission on the morning after admission (t. 100°), and several pigmented spherules and leucocytes seen; and next morning (t. 99·2°) I found numerous spheroids and crescents, and, in addition, many enormous endothelial cells, densely loaded with masses of black pigment, identical with such seen by me previously in a fatal case of febrile "melanæmia." On the same afternoon and next day, the pigment-masses had considerably diminished. On the third day after the cessation of fever, I found flagellate organisms; and on the two following days, spherules and crescents were seen in decreasing numbers, and the red corpuscles showed pale pigmented spots on their surface; then again the flagellate bodies were noticed, and next day, the seventh after defervescence, the blood appeared to be free of visible contamination of any kind: but on the ninth several very active flagellate bodies were detected: on and after the tenth day, for a further period of eighteen days, the blood showed daily some spotted red-discs in gradually decreasing number and degree.

7. *Continued* (*remittent*) *malarious fever*.—S. M., Mussulman, æt. 22, a mason from Jeypore, one month in Bombay (crowded locality) and twelve days ill with fever, was admitted 10th October in the incipient typhoid state, with t. 101·4° F. and spleen enlarged to two inches below the costal cartilages and very tender: signs of enteric fever were absent and stools seen were few, scanty, liquid yellow and alkaline, but not ammoniacal; urine scanty, high-coloured yet clear, sp. gr. 1012 and no albumen present. Quinine, in 10-grain doses, at three-hour intervals, being given thrice, pyrexia thenceforward declined, with some sweats, to 99·4° F. at midnight; the temperature further subsiding next day (quinine repeated), with abatement of splenic tumescence, yet a continued general depression, with clammy skin, reminding me forcibly of the state attending the lytic defervescence of the spirillar fever. Next day temperature rose to 99·6°, but declined again to normal on the two following days, quinine being repeated in smaller doses: the splenic signs were more persistent, and so marked as to suggest a local infarction: shallow fissures formed on the tongue, and a few days later some ear-ache, or possible parotiditis appeared, and about ten days after admission the indications of slight lobular pneumonia, on the left side above, and not conterminous with, the splenitis, resonant

lung intervening. During this period, and until his discharge, eight days later, the man seemed to rally very slowly; and though taking food fairly, he lost 10 lbs. in weight. Quinine with salines, and locally mild mercurial applications, were ordered. As compared with No. 6, the resemblance was at first considerable, but more pronounced were the marks of blood-contamination sequelar to the malarial: as, in that instance, the original infection seems to have been acquired outside Bombay, since the man was said to have had severe fever in his native town a month previous to his leaving for the capital.

The blood was for a fortnight carefully scrutinised twice daily, or oftener. Upon admission I found some crescents and spherules, with many deeply pigmented endothelial cells and leucocytes, and numerous intra-disc pigment-forms showing apparent transitions to free organisms. During the next four days aspects were similar, the dense melanic masses diminishing daily, crescents well formed, and with fewer of them on the 13th and 14th date. I noted several free small spheroids containing pigment particles in such active movement as suggested their recent connection with the flagellated state; but this condition was not actually witnessed until the 15th, or five days after full decline of fever. On the succeeding five days there were seen spherules, crescents, and pigmented leucocytes, with latterly some free indigo-tinted granules: ordinary pale cells were frequent in the blood and small plasm-clumps. With the slight pneumonic attack the organisms became fewer, and they finally disappeared about eleven days after the man's admission: in detail I could not connect their persistence and decline with any of the local or general symptoms contemporarily observed.

*II.—The Organisms.*—Their discoverer was of opinion that all forms pertain to a single "microbe," which is essentially free, and only at earliest stage attached (*accolé*) to the coloured blood-cells or discs (Laveran, *l. c.*, p. 168), the commonest aspect met with being that of detached pigmented spheroids: whilst later observers have laid more stress upon the intra-cellular site and frequency of the parasite. My own attention was drawn chiefly to the unmistakeable free bodies, but in the first positive observation made I recognised and figured those minute, and probably incipient, forms of organisms which are intimately associated with the red corpuscles.

*a. Blood-disc aspects.*—On concurrent testimony, these, if usually clear, may be so faint as to be obscure and scarce enough to be overlooked. I note further that, as sources of possible illusion, flaws in the glasses and the intrusion of foreign particles need discrimination; as well as attachment to the red-discs of free plasmic molecules, occasionally present in the liquor sanguinis. Again, it is well-known that the red corpuscles are themselves liable to incidental changes, not merely of contour but throughout; and in this tropical climate I find after intervals of watching that normal discs (viewed in control-preparations) are susceptible of alterations in stroma and tinted plasma which resemble localised condensations or rarefactions, moving as if amæboid; a common aspect being that of pale reddish spots of variable number and shape, surrounded by a turgid greenish border (*vide* Plate IX A, Fig. 7): and in common febrile states, red-disc changes, apparently both physical and vital, have seemed to me especially frequent and puzzling (*vide* "Spirillum Fever," London 1882, p. 341). In the cases under review

appearances similar to the above may all be met with; and, in addition, I noticed others probably unconnected with the pigmented bodies, such as active pale coccus-like granules, and even more striking heavings of the red-disc due to internal amæba-like masses (*vide* Plate IX A, Figs. 5 and 6). None of these aspects are attended with actual pigmentation, and if the formation of dark granules within or from the hæmoglobin of the discs be regarded as a mark of the malaria-organism, the following would be the more characteristic:—(*a*) minute, pale, rounded spaces, vacuolate or solid, which slowly change their form and site, and occasionally show dark spots (*vide* Plate IX A, Figs. 1 and 2); (*b*) pale-rounded spaces displaying within a dark pigment granule, which were seen in two or three hours gradually to expand over the area of the disc, the pigment-particles augmenting, actively moving, and finally disposed as in free crescent or spheroid. In one field, several red-discs were sometimes seen thus changing at various stages, as would appear, towards the formation of free organisms (*vide* Plate IX A, Figs. 3 and 4): not seldom the aspect was that of one or more small pigmented spherules imbedded in, or attached to, the red-discs, as was described by Dr. Laveran; and, upon review, it would certainly appear that a real connection exists between these changes in certain red-corpuscles and the isolated pigmented bodies. As my figures were made at cessation of fever, followed by convalescence and no increase of free organisms, I could only infer that they represent stages of arrested if normal growth; and opportunity is still wanting of tracing with certainty the completed transition of disc-change to free parasite.[1] Stained preparations, however, confirming this inference (*vide* B, Fig. 3), there seems ample reason to regard the pigmented spots on the surface of the red-corpuscles as not less characteristic of blood-contamination than the manifest detached organisms they precede and accompany. To show permanently the intra-cellular structures, staining of the blood should be slight or moderate, otherwise the pigment becomes obscured; and as, even then, tints seem often to be irregularly disposed, appearances may be deceptive.

*b. Free bodies.*—These are evident super-additions to the blood, dispersed in the common medium, and seemingly rather less mobile than the red-discs. They include distinct pigmented structures, of either globular or crescentic shape; and I may add also dotted plasmic specks, with irregular opaque particles of dark and bluish hue. The larger bodies attract notice by their hyaline colourless substance, contrasting strongly with the deep-hued opaque clumps imbedded in it. This substance is firm, elastic, more refrangible than the material of leucocytes, and resisting more the action of weak acids and alkalies; it seems denser on the exterior of both spherules and crescents, and, continuous with it are their flagellar and membrane appendages: it stains as readily, though not equally, with the aniline dyes. The pigment-constituent is invariable, as very minute

[1] Subsequent consecutive blood-scrutinies ahve strongly indicated such transition.

granules which are rounded, isolated, almost opaque, and of tint a deep orange-brown, in the aggregate seeming black: they are unaffected by drying and the ordinary re-agents, and when quiescent are arranged as clumps, streaks, or rings, of varying complexity. Less uniform and much larger masses of pigment ("melanine" so called) may be seen, either free or usually inclosed in leucocytes and endothelial scales: the particles of indigo hue were oftener free, but sometimes also enclosed: the structure of these coarser pigments seems colloid rather than crystalline.

1. The spheroidal or globular bodies were rather the commonest seen: their dimensions varyfrom about $\frac{1}{10,000}$ inch to $\frac{1}{3,000}$ inch ($2{\cdot}5\,\mu$ to $8{\cdot}5\,\mu$); contour defined, and aspect colourless bright and nearly clear, or slightly granular within: their shape round or ovoid, in the fresh state varying temporarily from slow amæboid movements, and apparent transitions towards the rod-form may also occur: they are either flagellated or bare. In ordinary specimens of fresh blood these bodies appear bare, and the smaller forms may always be so; the pigment-granules within, more or less numerous and clustered, presenting only a languid motion, irregular or intermitting, and gradually ceasing after some hours: but when of dimensions approaching the smaller blood-discs, and specially when displaying active pigmental movements internally, it is likely that the bare spherules had lately been flagellated. The number present in the field may be 1 to 6, or not more may be seen in the whole specimen of a hundred microscope-areas: there is no tendency to aggregate, but some adhesiveness is manifest in a clinging to the glass: a proper motive power seems absent. While preserving somewhat their contour on drying, it is doubtful if they possess a distinct coat or envelope; but, as would appear from the freedom with which the pigment-granules move within, the interior may be semi-diffluent and though not distinctly fibrillar yet possibly contractile, since such inner movements are often defined, regular, and intermittent for a short time, after which they cease with the assumption of a quasi-rigid and cadaveric aspect.

*Flagellated spherules.*—Must be common, if not invariable; since they were repeatedly seen in six out of the seven examples under review, and probably only just missed in the remaining case (No. 3). I never witnessed the advent of the flagella in connection with a pigmented globe, previously bare;[1] and always found their duration outside the body to be so brief that it is likely their presence was often overlooked by inevitable delay of a few minutes in searching. It seems that these motile appendages pertain to a clear plasmic investment of the central body, which is contractile, soft and evanescent. From their tendency to drag inwards the red-discs near in a cluster, the entire organism

[1] From a large and turgid bare spherule, with restless contents, flagella have latterly been seen quickly to emerge, lashing actively, and soon darting off as free active filaments; the central pigment-body then contracting in dimensions (fresh blood, post-febrile stage).

was rarely seen quite untrammeled; and the flagella being translucent, their number, arrangement, and length could seldom be clearly made out. Apparently 1 to 4 or 6 (or more?) might be present, radiating when several, and measuring about $\frac{1}{1,500}$ inch to $\frac{1}{800}$ inch ($17\ \mu$ to $32\ \mu$) long, with a mean breadth, thickest at the root but not tapering markedly, of about $\frac{1}{40,000}$ inch ($\cdot 6\mu$): an occasional knobbed aspect of the filaments might be due to folds or knots ensuing on whirling or lashing movements.[1] In the midst of the commotion thus arising—a striking sight to witness—I have seen a flagellum become detached, carrying off with it a fragment of the envelope, and flit to a short distance, where its presence was proved by a brief thrill amongst another neighbouring group of red-discs; and upon other occasions such isolated thrillings being seen, this phenomenon may not be rare. In the course of 10 to 30 minutes after withdrawal of the blood (seldom longer in the warm climate of Bombay), these cilia-like appendages and their matrix, would rather quickly disappear as if by dissolution, a state of momentary rigidity preceding; and the central body would then become somewhat contracted, sharply defined, and quiescent, having previously been tossed violently (without shifting a distance beyond, it may be, its own diameter) by the active flagellar action. At such time, however, the contained pigment-granules, also before intensely agitated, do not at once fall into repose; but continue for several minutes longer to circulate, often along definite lines of ring or band within the spherule; and such sustained active motion I came to regard as evidence of a late prior flagellated state of the spheroid, proof seldom appearing of its being a preliminary condition to such a state. The flagellated organism sometimes seemed to contain a clear nucleus in addition to the defined pigmented area; but this aspect was only temporary, and some illusion seemed possible: that the central mass was continuous appeared from the straying into the envelope of pigment granules, which afterwards returned; and its condensed sharply defined contour at close of the flagellate stage, together with rapid changes of pigment-pattern within, were noteworthy: no fresh alterations supervened after melting away of the flagella with their matrix, so long as the preparations were kept. The samples were generally much alike; but variations doubtless occur, and the specimen figured as No. 12 B, in Plate IX, seemed after gradual fading of the stiffened appendages to shrink into a pigmented plasmic mass, of less defined form than usual; a few ordinary spheroids were seen in the same preparation; and in next day's blood, still fewer of the same: pyrexia absent then and after.

Preservation of these ciliated bodies by drying and staining, was rarely accomplished satisfactorily; and out of at least 50 blood specimens made, in only

[1] Latterly I have seen filaments, both attached and free, which presented one or more distinct and translucent swellings, either midway or at end—*vide* Plate IX. Dr. Laveran also figured them: *l.c.*, p. 171.

two or three were the flagella detectible on close scrutiny. The bare spheroids take the dyes freely and often assume a granular aspect, which may be indicative of their structure: a distinct coat is not apparent in such and the dark pigment seems rather shrunken.

2. The rod-forms are curved and rather pointed at their ends, so as to be comparable to semi-lunar, crescentic, sickle or sausage-shapes; having their pigment-particles aggregated about the middle; and along their concavity, the traces of a thin arched membrane: forms transitional to the spheroidal, of varying length, may also be sometimes seen. Isolated and quiescent, these bodies float (Medusa-like) with the convexity upwards, and, therefore, show various degrees of curvature according to position: their length equals $\frac{1}{4,000}$ inch to $\frac{1}{25,000}$ inch (6 to 10 $\mu$), and their breadth the $\frac{1}{10,000}$ inch to $\frac{1}{8,000}$ inch (2·5 to 3·2 $\mu$); when seemingly entire and laterally viewed, the pendant membrane measures, at deepest, one to two times the breadth of the body, but it is usually shortened and shrunken, and even may not be visible: no cilium at either end was ever detected, and the free edge of the membrane is either smooth or jagged.

Crescent-organisms are common, and may persist alone for weeks, even after quinine; their contour is well-defined, consistence firm and elastic, and aspect clear or at most slightly granular: though not showing any amæboid changes of form, and seemingly without a distinct envelope on their rigid exterior, yet as occurs with the spherules above described, their pigment-contents commonly manifest for a short time after withdrawal of the blood, slow movements indicating a certain diffluence and activity of the internal substance; when quiescent after some hours, the pigment may appear as a folded membrane, or as aggregated or even scattered granules. Usually free, not unfrequently the crescents are seen apposed to side or edge of the red-discs; such contact may indicate a certain adhesive tendency possibly more than is manifest in the spherules, but it is not considerable, and as regards the larger organisms seems quite incidental: as an occasional aspect, I have noted crescents partially embraced in a folding of the discs,—*vide* Plate IX B, Fig. 2.

These bodies stain readily, yet not always uniformly in thicker parts, and in the membrane to only a slight tint.

In addition to the above, other free bodies seen were dotted plasmic specks; rare, minute, and noticeable chiefly in connection with disintegrating spheroids,—see below.

*Pigment free and enclosed.*—In a free state, dark particles, either large or small, were rare, being probably taken up by pale cells and conveyed away: such may be small, equal, and similar to the granules in organisms; or oftener large, unequal, and much coarser in aspect, specially when contained in endothelial cells,—see Plate IX B, Fig. 15: both forms occurring in close relationship of time with pigmented organisms, and bearing hardly less significance.

Coloured particles of an indigo-blue tint were not infrequent at later periods, and even for days after disappearance of organisms and of the melanoid pigment, to whichthey seem to be a sequel: their form was minute clumps, or plates, translucent even when of deep hue, and such materials were commonly free, being apparently little attractive to the leucocytes co-present.

*Life-history of the Organisms.*—That the parasitic forms above described pertain to a single species, seems highly probable from their common morphology, similar conditions of appearance, and usual concurrence or succession: yet the impossibility of tracing a continuous series of changes by inspection alone of the blood, indicates that the successive stages of development either do not take place in the general circulation at all, or else so obscurely as to elude detection. No doubt isolation and culture, apart of crescent or spheroid, might reveal the entire life-history of this polymorphic organism; and without such aid, only a surmise of events remains available. As to growth—germs are still unknown; but if pigmentation be a sufficient guide, it seems likely that the incipient formation of the parasite takes place in connection with certain red blood-discs, not at first of peculiar aspect yet now becoming tumid and bright, and showing a pale marginal spot in which dark, active, coccus-like molecules soon appear: such change advancing over the entire disc-area, a pigmented spheroid would result; or if extending over only one-half the area, a crescentic body whose membrane would then represent the uninvaded yet blanched segment of the disc. Evidence to this effect was, I think, met with particularly in Cases 6 and 7, upon long watching and repeated scrutinies of blood at short intervals (*vide* Plate IX A, Figs. 3 and 4); but I did not witness the completed transitions, as development seemed to be here arrested at close of pyrexia or under the influence of quinine. Doubtless, the organisms afterwards grow in dimensions (and probably, too, their pigment), a different course being followed: thus, the pigmented globes tend to assume the flagellate state, which can hardly, I think, last long in the blood and seems to be followed by segmentation, rupture and the dispersion of minute dotted plasmic specks ( ? germs), such as were occasionally seen free; whilst the crescents are more lasting, eventually, as was surmised, giving exit to a pigmented spherule or incipient organism (*vide* Plate IX B, Fig. 5). These conjectures are based upon upwards of 200 separate inspections of the blood, taken from native patients who might be neither feverish nor ailing much; the blood-droplets also not seeming abnormal, though charged with many parasites. Admitting, however, on the same evidence, the possibility of an independent origin in the *liquor sanguinis*, of crescents from spheroids, and of spherules from isolated plasma-specks, a difficulty ensues to interpret aright the direction of changes, since retrogression was under the circumstances at least as possible as evolution. Watched under favourable conditions for 24 or 48 hours, no late alterations beyond those of decay, were

seen in either sphere or sickle; and, much earlier, the intra-disc aspects lost their definiteness, without advance.

Regarding the parasitism as a whole, nothing definite has yet been learnt of the mode or time of the first introduction of germs: though, according to current views, the malarial infection can be acquired through both air and water, and this research would certainly suggest the channel of drinking-water by preference. Its prolonged duration, also, sustained by means of repeated periodic exacerbations, is a phenomenon now rendered explicable: and assuming still a relationship of organism to infection, it becomes interesting next to note a particular way in which there was brought about a subsidence of visible blood-contamination, so invariable as to suggest the epithet of 'natural.' I refer to the active voracity of leucocytes in the blood (the larger coarse-grained kind especially), seen at all temperatures equally whenever flagellated spheroids were present: this circumstance proving a positive hindrance to continued observation of the organisms, which seemed to constitute a pabulum so attractive as speedily, in some occult way, to draw into the field one, two, or even three of the amæbæ eager to contend for the prize. The most violent and repelling contortions of flagella were then, I saw, of no avail in opposing the overwhelming advance of expanded end (mouth, so to speak) of the phagocyte; and always in the course of a few minutes (extending to 15 or more) the entire pigmented body became engulfed, and was carried off to be digested at leisure; leaving at last only dark pigment-granules, such as were not uncommon in many leucocytes, and might hence serve as evidence of prior characteristic events. In Plate IX some delineations are offered, and a sample of two spherules within one amæba. It further appeared that if in this way the active spheroids are commonly removed, their transitory duration being thus accounted for; yet, for some reason, the crescentic bodies were not so attractive to marauding phagocytes, which I repeatedly saw to turn from, and leave untouched, these tougher organisms when encountered in their wanderings, and, such being the rule, the longer persistence of crescents in the blood may also be hereby partly explained.

In every one of the seven cases studied, the amæba-warfare was seen and figured, and often repeatedly: no sign appeared of the spheroids and their flagella being able to protect themselves against attack; and after involution with disappearance of all but the rounded central body, I frequently saw the pigment-granules move definitely, as if disintegration of the spheroid were delayed; the idea occurring that germ-granules might be even preserved latent within the leucocytes, for a certain period of time.[1]

*Provisional Identifications.*—The structures above described are termed "organisms" from their constantly definite aspects and relations, and their

[1] Latterly the above observations have been exactly repeated, and the surmise gained that currents in the plasma may convey to the phagocyte intelligence of its more or less distant prey. Not all spheroids are equally attacked, or else not all amæbæ are equally eager.

resemblance to other bodies always recognised as such. Further, on account of their non-cellular character, quick transitional and motile changes, they should, I think, be regarded as "hæmatozoa" rather than "hæmatophyta": and, in particular, they might be referred to the parasitic "monads" amongst Infusoria, nearest perhaps to the *Trichomonas sanguinis* of the rat and equines elsewhere alluded to in this volume. Yet the organism of ague has peculiarities of its own and may be more than generically diverse, its comportment at the flagellar stage departing widely, and the resting-stage, as represented by the crescentic bodies, being singularly predominant: in a sense, such curved bodies recall the sickle-shaped germs of Coccidium (of the class Sporozoa immediately below Infusoria), which, as *C. oviforme,* parasitically, infests the liver and intestines of mice and several domestic animals, not sparing man himself. This is mentioned because there are indications that several blood-infections of man causing specific disease, originate in parasites primarily lodged in the tissues and there harmless.

That at present in the blood these malaria-organisms are essentially foreign, will be evident: the bright hyaline material of the spheres may offer a certain resemblance to that of leucocytes and be likewise (if less in degree) amæboid, yet their pigmentation is well-nigh characteristic, and the flagellate stage even more so: the crescents from their aspect alone are quite distinct from any normal blood-structure. In specimens of fresh blood, the spiral bacterium of man, the flagellated monad of the rat, and that of the human ague-blood, all cause by their movements appearances of commotion which it might be difficult at first sight to discriminate; but yet briefly, for after a few minutes the pigmented spheroid loses its appendages for good, the organism of the rat endures for only a few hours, whilst the bacterium, becoming quiescent, within the same period commences to grow in the blood and may be preserved for some days.

Lastly, I would note the morphological and biological identity of the ague-organism, as depicted by its discoverer Dr. Laveran in Algeria and Italy, by Dr. Osler in the United States of America, and by myself at Bombay. Though unable at present to insist upon the significance of minute blood-disc changes so much as authorities elsewhere do; yet an expression of my own agreement so far should not be omitted, as fresh testimony upholding the distinctive characters widely displayed by the malaria-infection.

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NOTE.—After the above had been written out for press, I met with an analysis in English of a paper on the Microbe of Malaria and its Relation to Phagocytes by Professor Ilia I. Metchnikoff (*sic*) of Odessa, which is published as No. 7372 in the LONDON MEDICAL RECORD of 15th October 1887, p. 468, and which contains the following items of comparative interest here:—

"The red blood-disc changes are freely recognised: discs with the larger intra-cellular spheroids do not take the eosine stain. Segmentation, or breaking-up, of the spheroids within the red corpuscles is described, and it is considered that the small daughter-spheroids resulting then become free, and enter into other blood-corpuscles."

(*N.B.*—The bodies in Plate IX B, Figs. 7 to 10, probably correspond to the so-called 'rosette,' or 'daisy,' stages in the evolution of pigmented spheroids, as described by authors in Europe.—H. V. C.)

"As regards identifications Professor Metschnikoff analysing previous investigations, 'comes to the conclusion 'that the parasite of malaria belongs to the group of *Coccidia*, to the genus *Hæmatophyllum*, which is pretty closely

'allied to another genus of the same group, called *Klossia* (as well as to V. Danielevsky's *Trypano-Somata*). As the 'author thinks, *Hæmatophyllum Malariæ*, like the *Coccidium oviforme* of the rabbit's liver, does not develop any 'resting-spores within the living organism. Probably the daughter-spheroids do not transform in the human body 'into sporoblasts, but only give generations of amæboid bodies and encysted forms (crescents), which spread over 'the blood of the individual. The spores are generated and ripened only after a prolonged sojourn in the soil. 'Such a supposition might explain a pronounced miasmatic nature of the malarial virus, which can be inoculated 'solely by way of a direct introduction into the blood,' p. 469."

(*N.B.*—The allusion to Coccidium shows that the comparison in the text, had occurred to others than myself. No mention is made here of the flagellated organisms. The next remarks refer to 'phagocytes', whose wide influence in pathology Professor Metschnikoff has long forcibly upheld.—H. V. C.)

"In the spleen of a patient dying of malarial meningitis, there were found only a few microphagi (leucocytes) containing red blood-corpuscles with the microbes, and masses of a dark-brown pigment; but a great number of macrophagi (large cells of the splenic pulp), containing red blood-corpuscles in various stages of dissolution and coccidia in early evolution-forms, as well as isolated heaps of melanine (black pigment) and smaller granules of pigment. Similar macrophagi were abundant in the liver of this and another patient. Hence the author concludes that 'the human organism struggles against the malarial microbe with help of devouring cells, mainly with help of the macrophagi.'"

(*N.B.*—Leveran had previously made similar *post-mortem* observations, *l. c.*, p. 59. The bodies in Plate IX B. Fig. 15, doubtless correspond to the above, see also a coloured drawing of the hepatic blood from a fatal case of malarial melanæmia recorded in Trans. Med. Phys. Soc. of Bombay No. VIII, New Series, 1886 p. 30.—H. V. C.)

"Some facts (concerning partly malarial, partly relapsing fever) led Professor Metschnikoff to believe 'that the malarial parasite in its *free* state is not devoured by phagocytes, possibly in consequence of its excreting a *succus* on its surface, which, as it were, paralyses the phagocyte. Hence its proliferation in the blood may proceed uninterruptedly, to a certain extent, but with its entrance into the red blood-corpuscle the microbe loses its power of resistance to phagocytes, and then the latter, which generally devour 'enfeebled' red corpuscles in the human body, begin to devour also the corpuscles enfeebled by the coccidia.'"

(*N.B.*—The Bombay observations demonstrate an amæboid activity which at Odessa could not have been known; and they also disprove any notion that even a multitude of organisms in the blood necessarily entails the febrile state. Reference being here made to relapsing fever, I desire to point out that in 1878 I had watched the destruction of a spirillum by a leucocyte, and figured and described the phenomenon, which is alluded to at page 366 of my work on Spirillum Fever, London, 1882. It may be added that Professor Metschnikoff has shown, by experiment on the monkey, that spirilla thus taken up by leucocytes still retained their infecting power; and this fact, with the occasional free appearance of the spirilla, is considered to explain the recurrency of the febrile attacks, —*vide* LOND. MED. REC., October 1887, para. 7369.). According to this view, the relapsing tendency of both spirillum and malaria-fever would be similarly explicable.—H. V. C.)

3. *Relations of the Organisms.*—These, as inferred from the foregoing data, may be ranged as follows:—

Comparative frequency of the pigmented bodies. My 7 cases were examined once, twice, or oftener on 127 days, the free crescents being noted on 72, bare spheroids on 68, and the flagellated on 20 days; undoubted pigmented spots on or within the red blood-discs were not seen nearly so often as the free organisms. These results are too contingent to have more than a limited value; yet with them may be usefully compared the experience of Laveran, who in 432 cases found crescents 107 times, spherules either free or sessile 389 times and the flagellate bodies 92 times, adding that often the red corpuscles present small clear spots which are probably incipient spheroids; whilst, on the other hand, Osler in 70 cases ascertained that the most common alteration in the blood of malarial patients is presented by a pigmented structure inside the red corpuscle, finding the crescents in 18 cases only, rosette-forms (? spheroids) in 8, and flagellated organisms in 7. There hence appears a certain discrepancy which remains for explanation; and doubtless if the observations on blood-disc changes

made in Italy, more especially, become confirmed, such indications will be more insisted on here, where as yet experience has led me to hesitate in adopting all their reputed specificity and pathological significance.

Seemingly, the consistence and tint of blood-specimens, bears no fixed relation to the organisms present in the circulation.

As regards relations to the general state of patients' health, although it be conceivable that some kind or degree of occasional blood-parasitism may seem as innocuous in man as, *e.g.*, in the rat, still blood which visibly contains a large number of foreign organisms cannot correctly be termed healthy; and the question of time needful for manifestation of symptoms being here important, I would remark that, in the present instance, an early history of more or less febrile illness has always been available; and without some evidence of disorder, either prior or actual (it might also follow), the organisms were never seen.

Adverting to their possible special morbid significance, I note further, that it was solely in connection with signs of the so-called "malaria" infection, that these bodies were found; and on many trials, never in connection with other common diseases, either febrile or non-febrile,—see the List B above, to which spirillum fever may surely be added: my experience so far, therefore, agreeing with the testimony of Professors Laveran and Osler.

Enquiring now into the particular evidence of such relationship, it becomes apparent that this instance is more complex than the majority of specific blood-diseases; the data showing, on the one hand, that the malarial infection is compounded clinically of both acute and chronic phenomena; and on the other hand, that the visible blood-contamination varies both in itself and in its association with symptoms and treatment. Thus, in detail, febrile exacerbations being regarded as the more characteristic phenomena, the case first seen—No. 1 quartan ague, served to prove that, simultaneously with each exacerbation, there occurred the advent of organisms both intra-cellular and free (as bare spherules), and that when fever was arrested by quinine, still organisms re-appeared on the next periodic date, but then flagellated; after which event, quinine being continued, the blood seemed quite free during 15 days' longer observation. Case 2 was one of old quotidian ague, in which for 11 days, along with daily paroxysms, spherules and crescents were present in the blood; upon arrest of fever by quinine and continued administration of the drug, these organisms still persisted, flagellate bodies being also seen on the 4th day of apyrexia, and for 7 days longer. Case 3 proved that crescents may persist in the blood for 11 days after spontaneous subsidence of fever; and consentaneously with the onset of fresh febrile paroxysms, intermittent in type, they may practically disappear; re-appearing with arrest of fever by quinine, now first given and continued, and persisting in fewer numbers for 21 days; some spheroids and probably flagellate bodies being also present on second and third day after checking of the intercurrent pyrexia

case 4 of old quotidian ague, demonstrated the occurrence of free organisms at least, with apyrexia of 5 days' duration; and with the onset of fresh intermittent attacks, their persistence with the occasional addition of flagellate organisms for other 9 days' observation, suddenly closed: quinine not given. Case 5 presented the phenomenon of continuous complete apyrexia for at least 42 days, during the first 15 of which the blood was examined repeatedly on 10 days, and found always to contain numerous crescents and spherules, bare and flagellate, no quinine being given: after a brief exhibition in total of 20 grains of this drug, the organisms began to diminish, the flagellate not being seen, but the other forms continuing for 15 days longer, after which the blood was clear for 11 days more: how long before his admission this patient had been fever-free, was uncertain; his only complaint in hospital was splenic enlargement and uneasiness, which had not quite subsided when he left. Cases 6 and 7 were much alike, both being examples of ague of severe continued (? remittent) type; at the time of pyrexial arrest by quinine, abundant organisms in the blood, and, in addition, pigment-loaded cells, both leucocytal and endothelial: after continued exhibition of the drug, these bodies subsided in the course of 11 or 12 days and; in both cases, shortly after the arrest of fever (3 or 4 days), flagellate organisms were for the first time seen; in No. 6 three times in 6 days, and in No. 7 once only: following which, the blood seemed healthy for 17 and 7 days' stay respectively. This review is offered because, after allowing for defects, the data appear adequate for determining the fundamental question of visible relationship of organism to pyrexia and other disturbance of the system; and it demonstrates the absence of a direct connection between the phenomena compared, as regards actual concurrence in time (*a*), kind of organism to kind or severity of fever (*b*) or to other general affection of the system (*c*). So far as I learn, these results are in main accord with those arrived at elsewhere; and they render it needless to discuss the precise relation of special organism to special symptom, because already proving the absence of such relation.[1] This is not to assert the impossibility of otherwise demonstrating a general or special connection, for, judging by other experience, *e.g.*, the spirillar, some such is likely; and even here, if the morphological data be allowed to include excessive splenic or hepatic pigment-formation, very serious symptoms (as in cases 6 and 7) may be seen to happen simultaneously.

*Diagnostic imports of the visible Blood-state.*—As hitherto estimated by history and symptoms, the diagnosis of the malarial infection is so often obscure that clinicians would welcome any additional aid afforded by microscopical scrutiny of the blood. This obscurity in diagnosis being attributable mainly to the chronicity and variableness of clinical signs, it might *a priori* be anticipated that contemporary blood-states, as now revealed, would vary likewise; and, as matter of fact, it is found that the monad-parasite of ague

[1] Later experience has confirmed these inferences: see the Postscript at end.

not only displays wider polymorphic aspects, but also maintains a relationship to symptoms so indirect as to render its significance both restricted and peculiar, as compared with other infecting-organisms of either acute fever (*e.g.*, the spirillum) or chronic disease (bacillus). It is unfortunate, so to speak, that in malarious disorders some febrile periods should prove dubious in their blood-signs ; since a valid test was needed for analysis of a common fever-type here, sometimes called the "typho-malarial." My experience, hitherto, is conveyed in the two lists above (p. 140) : and Dr. Laveran states that he found the microbe of paludism disappear with onset of a 'typhoid' complication, at its close re-appearing with the return of ague : doubtless prolonged research is needed, and will be made. Regarding the blood-manifestations of a primary malarial infection, enquiry at Bombay was not practicable ; in Algeria Dr. Laveran found the microbe identical at both first and recurring attacks of ague : information is required regarding changes in the red blood-corpuscles, at first.

Subsequently, it is conceivable that, with infection for a time latent and quiescent, no other sign might be available than some lasting visceral lesion earlier arising, and it is true that at Bombay the microscrope frequently failed to supplement the ordinary means of diagnosis ; yet upon review, I think it probable such failure was incidental, and that an infection not abrogated will always be detectible through its attendant blood-changes. In practice, positive aids to diagnosis were as follows:—(*a.*) *Free pigmented organisms ;* namely, spherules bare or flagellate, and the crescents : both these forms being pathognomonic, a single parasite of either, once detected, becomes decisive evidence of a malariou staint, whatever the attendant symptoms. Yet with such taint, no fixed elation appears of form of organism with kind of symptom ; and free organisms were absent in my cases at apyretic intervals (No. 1), and even during some intercurrent paroxysms (No. 3), no quinine being yet given : hence their non-detection, under some conditions, has no absolute clinical significance. Absence may be difficult to prove, and as yet the elements of certainty are wanting. The minuter pigmented plasma-particles which were probably incipient organisms, did not occur alone.—(*b.*) *Altered blood-corpuscles.* 1. Red-discs displaying on their surface one or more pallid, pigmented spots, moving and growing, are decisive affirmative evidence, and were seen in case 1 (pre-febrile), and case 7 (post-febrile period), along with free parasites. It is stated that they may occur alone, as sole sign of infection ; being the nregarded, in even scanty numbers, as valid testimony : and according to some European authorities, they are constant enough to serve as a means of diagnosis of actual malarial agency. These data have seemed, at Bombay, to need some qualification, a full verification at least not being attained. Red-discs thus altered appear to represent an early stage of the free organisms, but may not be traceable

onwards into such (cases 6 and 7). 2. Leucocytes containing outside their nucleus black pigment-granules, few or several, having been seen in connection with free organisms and not occurring in other infections, constitute, even when alone, decisive evidence of malarial blood-deterioration: they are late phenomena, and may persist for some days; but they were not always visible, even concurrently with other evidence, microscopic and clinical.—(*c.*) *Much larger pale nucleated cells;* ladened with pigment (melanine) in the form of clumps, extra-nuclear; often large and numerous, colloid rather than crystalline, and unaccompanied with disintegrating red-discs, were in my experience rare yet quite characteristic (cases 6 and 7): concurring with free and sessile organisms, some persisting longer and a few for a day or two alone: they are derived from the splenic pulp and endothelium. (*d.*) Pigment in free state; black, brown or blue in tint, having been found in the blood as late and final stage of veritable infection, bears a certain valid significance: such particles very scantily and alone seen in a few malarious cases under List A above, were not, however, regarded as proof enough.

*Prognostic import.*—The natural, unopposed course of the malarial infection seems highly varied; being acute, sub-acute, or chronic, and either early fatal or under certain conditions spontaneously subsiding. Owing to the beneficial action of an accessible remedy—quinine,—prognosis becomes more favourable the clearer the diagnosis. With one exception, the visible blood-states have not proved of particular prognostic value: thus, in case 5 organisms flourished in the blood without any concomitant fever or even discomfort; and No. 3 showed their disappearance temporarily during the occurrence of smart intercurrent pyrexia; the incompatibility of blood-parasite and pyrexia being also indicated in No. 1, which displayed abundant spherules in the cold stage, and fewer and disintegrated organisms as the acme of fever was approached and passed; leaving no trace during apyretic intervals, except once, on date of a periodic suppressed relapse. The flagellated bodies were never present along with pyrexia: it has been said that they precede febrile onset (Laveran); yet after quinine at Bombay they were rather terminal phenomena.[1] Case No. 2 showed, repeatedly, smart fever (t. 102-3° F.), with many crescentic organs, in the blood, and no suffering (quinine not yet given); so that probably febrile reaction is really a contingent event. As regards the pigmented intra-disc forms, it was proved by Nos. 6 and 7 that a considerable number of red copuscles may be undergoing invasion and disintegration, at a post-febrile period (quinine given) while convalescence is steadily progressing; and this datum I regard as important. To what extent malarial fever is self-limited, has yet to be ascertained, see the next heading; several cases of prolonged fever here, known as

[1] Tertian ague ending spontaneously has lately displayed many flagellate organisms for several days, beginning with the third after cessation of fever: so that the influence of quinine is not essential to this remarkable phenomena.

"typho-malarial" or "remittent," have been scrutinised in vain for pigment organisms in the blood, either sessile or free. It appears that other febrile re-action than the self-generated is capable of checking or interrupting the growth of malarial blood-organisms; such as symptomatic fevers and the enteric, so long as they persist, the parasites re-appearing subsequently. The blood-state of special significance alluded to above is that termed "melanæmia," and such as presented by my Nos. 6 and 7, both of which were in a critical state on admission and might have died: the minute aspect of the blood was remarkable (see Plate IX, the last figure), and nearly as pronounced as in a fatal case of "fever" acquired in Northern India, which I have elsewhere described.

*Therapy.*—The high repute of quinine as an antiperiodic remedy (as well as antipyretic) in malarious fevers, was fairly confirmed here: thus, case 4 having no quinine and No. 5 no fever, in Nos. 1, 2, 3, 6, and 7 the specific pyrexia was promptly, completely and permanently arrested by this drug in 10-grain doses, repeated once or twice at close of defervescence, and thence at short intervals in smaller doses daily: simultaneously with such effect the splenic tumescence subsiding, and general health improving. As to the *modus operandi* of quinine in possible connection with the blood-organisms of ague, my observations were clinical only; direct experiment on blood outside the body, not (as in the spirillum-contagion) seeming of much real import: and hence in the absence of a definite visible relationship of parasite and pyrexia (see above), it was not easy to make a satisfactory inference from the data. Case 4 may be viewed as a sort of standard; in the absence of the drug fever persisting though declining, and the blood-contamination remaining unabated; the spleen meanwhile lessening an inch transversely, and the health improving. No. 5 had manifest blood-infection, without the least degree of fever; after verifying these points, two 10-grain doses of quinine were given on successive days; when the body temperature still continued normal and unchanged; whilst the blood-organisms began gradually to diminish, the flagellate disappearing on or after the third day, by the seventeenth the spherules, and by the twenty-second the crescentic bodies being no longer visible: here it seemed that a small quantity of quinine had led to a gradual suppression of the organisms. The remaining five cases, including mild and severe illnesses, had, after a preliminary watching, quinine administered to about 20 grains daily, and invariably with the result of a prompt and permanent arrest of the pyrexia; but not of a simultaneously quick disappearance of the blood-parasites, which on the contrary continued to be visible for periods of 4 days (No. 1) to 19 days (No. 3), or possibly longer, after the cessation of fever, during which time the drug was still being given. In the severer cases, 6 and 7, I noted that for 6 or 8 days after arrest of fever, there was still an abundance of pigmented blood-discs; looking as if a partial or quasi-abortive development of the organisms were going on, some free organisms being also present: and probably

a similar abortive growth took place in the other milder cases after cessation of pyrexia, and concurrent with the continued exhibition of quinine. The fact was also elicited that in every one of these five cases there happened from 2 to 5 days after the artificially induced crisis, a development of flagellated organisms in the blood, either once or repeated at 2 or 3 days' intervals, after which the visible blood-contamination might soon end. The total amount of quinine exhibited in individual cases was from 60 to 280 grains: always 20 grains seemed enough to stop the fever, after which events varied in detail. The above facts indicate that whilst the alkaloid arrested the fever at once, it effects this by some special agency not connected with the hæmatozoa in their visible aspect; these bodies still persisting unchanged for a time, after the cessation of pyrexia. I would further note that the voracity of the blood phagocytes in their eager pursuit of the pigmented spheroids, flagellate and bare, seemed in no way impaired after full doses of quinine given by mouth, or by the sub-normal body-temperature thereby often induced: indeed, it appeared as if evolution of the flagellated stage had, as its accompaniment, an increased activity of the amæba; and possibly the depurative action of the alkaloid might be explained by its stimulating rather than paralysing the phagocytes. The empirical recommendations to give quinine at certain hours before or after the febrile paroxysms of ague, or during apyrexia on certain days of expected relapse, would receive both support and explanation, were the hypothesis of a direct relation between organism and paroxysm sufficiently established on other evidence; but at Bombay it did not seem that a relationship close enough to apply here could be maintained.

*Pathogeny.*—The following remarks are based on personal observation of malarial disease, and of the analogous monad and spirillar infections of man and animals. The organism of ague as above described, being morphologically identical with that seen in the West; its import, biological and clinical, would also be recognisable here. Respecting parasitic blood-diseases even long investigated, opinions often differ as to character, source, and properties of the organisms concerned; and as regards the malarial infection, studied anew, divergence seems not less inevitable, with possibly some disturbance of general views held as settled. Difficulties pertain especially, I think, to the character of the parasite (*a*), and to the complicated symptoms of its associated disease (*b*).

(*a*) A foreign organism belonging to the animal kingdom might, in the vertebrate host, have effects different from those attending the common plant-infections; for, as remarked elsewhere, the hæmatophyta, as less germane, may be less tolerated than hæmatozoa; nor do I recollect any example of bacterial blood-contamination so abundant and continuous, and yet so apparently harmless, as the monad-infection of the common rat. This instance has been chosen for comparison, because an apparently identical infection of equines being highly virulent, it serves to show that an infecting organism may be either pathogenous

or non-pathogenous according to circumstances; and a chief discrepancy revealed in the present research as needing elucidation, is, I consider, the striking separation of virus and symptoms shown in the cases detailed. That a plentiful monad-infection should give rise to pronounced, and possibly periodic, disturbance of the system, is sufficiently comprehensible; and it was the partial disconnection of the phenomena, which seemed to be obscure,—see especially case No. 5. The explanation suggested of the trichomonous anomaly was that the organism of the rat has different properties from that of the horse or mule; possessing, that is without change of aspect, a biological diversity, such as can be demonstrated to arise in the bacillus of anthrax under certain conditions of growth. The fact of such occult change in properties being possible, however interpreted, warrants the inference that sometimes, *e.g.*, in ague, the parasite may within the same host undergo beneficial modifications; then under certain intervening condition alone giving rise to fever, whilst, in general, operating as a more or less wasting agency. This question is, I consider, not one of a mere co-temporary junction of phenomena; for if intervening conditions be thus effective (direct destruction of blood-organisms by drugs being impracticable), the attempt to cure 'malarial fever' might be made through means of them; and, in point of fact, I have shown that quinine does operate in such way, arresting pyrexia at once without immediately eradicating the organisms; or even without preventing their continued development, until after an interval of time long enough to prove its comparatively slow and indirect influence on their growth. Upon trial, Fowler's solution of arsenic proved inefficacious in either arresting fever (case No. 1), or destroying the parasite (No. 5).

Amongst the conditions unfavourable to persistence of organisms, may be the more pronounced pyrexial state belonging to the infection, *e.g.*, a body temperature of 104° F. (40° C.), as was indicated during the quartan paroxysms of case No. 1, also during the intercurrent paroxysms of No. 3; in No. 2, however, organisms were not lessened at 103° F., nor were they numerous during an abortive recurrence with low temperature in No. 1: in none of these instances had quinine yet been given.

Doubtless the visible blood-contamination may naturally subside, though under what conditions and if at definite periods, has yet to be ascertained; latency or apparent disappearance of organisms from the general circulation does not, it seems, imply their entire absence; for remnants, or germs, persist in the splenic pulp-cells (Laveran and Metschnikoff), which it is conceived may, by after development or increase, either spontaneous or incidentally aroused, give rise to renewed outward manifestations, either hæmatic or systemic.

The *modus operandi* of organisms in either fever-production or self-limitation, remains as little known here as in other acute specific infections: my enquiries were not directed towards the subject of ptomaines, or virulent meta-

bolic bye-products of the parasites. Phagocytes did not, for some hours at least, seem at all inconvenienced by the spherules they so eagerly engulfed, even when flagellated.

A correct identification of the ague-organism is desirable, if only as a clue to its detection outside the human body, and thereby readier eradication from the environs of dwellings. Of late it has been successively termed an alga, a fungus, sporozoon, and infusorium ; if rightly the latter, water or a liquid medium would obviously be needed for its growth and would serve for common vehicle ; in the state of sporo-cyst desication might be survived, and conveyance by dry air-currents : dissemination by evaporation would be unlikely, less so transit in solid vapour-particles. Hitherto, the organism has not been recognised outside the human body ; yet, being highly polymorphic, it might need to pass some stage of development in the exterior, possibly in certain soils or in stagnant water, under the influence of free organic nutriment, heat, and moisture. Contagia from without enter the frame by various channels, and, multiplying, are dispersed by lymph or blood-channels ; so operating, possibly other organisms besides cocci and germs or spores, as bacilli through ingesta and by way of lymph-follicles in the mucosa of the ileum. Expanding such notion, even infusoria from without, common and innocuous in the intestinal canal and ducts, might under modifying circumstances find a passage into the circulation and there display a certain virulence. And not to speculate further, it seems to me that the efficacy of measures for the removal or prevention of 'malaria' and the avoidance of infection, as ordinarily practised, is rendered not less intelligible than before but more so, by the demonstration in 'ague' of a seemingly pathogenic hæmatozoon, morphologically allied to organisms which exteriorly flourish under mal-hygienic conditions remediable by drainage, cleanliness and ventilation. Elementary animal forms abounding far less than rudimentary plants, amongst them the pathogenetic species (always exceptional) must be comparatively very rare ; their effects on man and equines (*e.g.*) being, however, not dissimilar to those of some bacterial infections, as regards general features of persistency and latency, with a tendency to periodic exacerbations under exciting conditions alone as yet controllable therapeutically.

(*b*) Doubtless, the malarial infection maintains the same inclusive characters in both East and West ; but whilst in Europe the discrimination of other concurrent infections has freely advanced, in India there holds still the conception of malaria as a well-nigh sole and universal cause of fever and cachexia. Upon review of my data, comparative and actual, I note that the manifestations of malaria are marked by a long intermitting persistence, with a localisation limited and but little irritative : thereby differing severally from those of typhus, typhoid and spirillar amongst the acute, and from tuberculosis, syphilis, and leprosis amongst the chronic infections of man. Protozoic human blood-

contaminations being hitherto unknown, it has been impossible to conjecture how such might differ in detail from the bacterial. A hematoid (filarial) blood-contamination shows, indeed, the tendency to persist with exacerbating intervals, but its nature and parasitism are essentially diverse; and a fit comparison can be made only with the trichomonous infection of equines, described elsewhere as being attended with wasting, recurrent pyrexia, and occasional lesion limited to the vascular area proper. Study of the spirillum fever led me to remark that that pyrexial symptoms, clinically insisted on, are really more contingent than the visible blood-state; and this statement will apply, upon evidence submitted, even more forcibly, to the malarial disease. The datum seems, indeed, to be comprehensive, and accordingly 'ague' may be defined as a zooic blood-contamination *prope cum febre:* pyrexial events, however prominent, being but subordinate incidents, whose conditions of occurrence become the proper subject of curative medicine: biology here blending, as it were, with pathology and therapeutics.

In the absence of autopsies, I have no fresh information respecting the fundamental splenic and hepatic lesions of the malarial infection: from other sources (*vide* Note above, p. 152) it appears that pigmented organisms, free and involved within phagocytes, accumulate in the organs named; and their power of reproduction persisting, thence a source of future growths and clinical manifestations. Such accumulation seems to be a special febrile concomitant, attended with vascular turgescence and changes clinically detectible: for at non-febrile periods organisms visibly abounded in the blood for days, without the abdominal organs being unusually swollen or tender. Particular implication, in ague, of the spleen and liver, may be due mainly to their office in the formation and destruction of blood-corpuscles, which here are the peculiar site of parasitic infection: yet it is noteworthy that the lymphatic system of glands, even the bronchial, is not simultaneously excited; and hence a question if entry of the infecting agent by the *prima via* and portal venous apparatus be not also indicated here (and in some other acute infections) by such predominant irritation of certain abdominal organs.

The production, in ague, of dark pigment seems indicative, not only of a special character of the hæmatozoa, but also of a concurrent metabolism extrinsic to them. Within organisms, the minute pigment-particles appear very early, and move and multiply so quickly as almost to suggest their living nature: enlarging somewhat, their regular form is maintained with an activity more evidently imparted; and thence in ordinary cases, no more is seen than the ready disappearance of pigment along with other marks of blood-contamination. But in severe attacks the formation of 'melanine' is so copious as to lead to the production of comparatively large, opaque, irregular or angular (or quasi-crystalline) masses, which impart a striking aspect to the leucocyte or

spleen-pulp cell in which they are imbedded. Such masses were so seldom seen free that their formation in the *liquor sanguinis* appeared doubtful; and I considered it more likely that such densely pigmented cells belong to the spleen, from thence entering the circulation where their presence may, it is held, lead to embolisms of serious or even fatal degree. It appeared to me physically impossible that these large pigment-clumps were formed by aggregation of particles derived from dead parasites; and I inferred that even if, as they seem, they be of the same material, their origin was a common and concurrent one from hæmoglobin: in the spleen each of such pulp-cells taking up and destroying numerous infected red blood-corpuscles. The data now mentioned are worthy of note, and they also serve to confirm the opinion expressed above, that the collateral events of visible infection, co-temporarily occurring, may be of chiefest clinical import.

*Secondary fever.*—The spirillar investigation (Work, pp. 171 and 420) led me to recognise pyrexial states following closely on the specific, and similar enough to permit of confusion, which are yet distinct in not presenting the same visible blood-infection; and I considered such consecutive, residual or sequelar pyrexia to be the effect of secondary quasi-septic contamination, or possibly of a re-action of the nervous system. Demonstrated so far for the typical spirillar, secondary events of this kind may be usual in other infections; and in the malarial, they appear to be represented by the so-called amphibolic stage of ague, long since noted by Wunderlich and his English commentators. At the native hospitals here, confirmed malarious subjects are seen presenting smart, yet not distressing febrile paroxysms, samples of which in my hands seemed unattended with pigmented blood-organisms, and therefore were regarded as of such secondary character: care being, however, needed to distinguish them from negative intercurrent paroxysms in the visibly infected, such as witnessed in case No. 3. How far the more prolonged fever of equally idiopathic aspect, which is often seen in malarious subjects, can be viewed as of this secondary, or sequelar, character remains for the present undecided; the spleen may be moderately involved, but the blood contains no pigment organisms; and quinine has no peculiar efficacy; signs of enteric fever are absent. This class of cases represent one form of the so-called 'malarial remittents,' which amongst natives take the place of 'continued fevers,' so-termed in rather antiquated phrase; and, upon review, the data at my disposal indicate that in such cases the absence of visible dotted parasites is not warrant enough to negative an antecedent connection, at least with genuine malarial infection.

POSTSCRIPT.—It has been possible to review the above statements with aid of other cases, 20 in number and, as more selected examples of malarial fever, furnishing 6 instances of the distincter blood-parasitism (not alone pigmental), which closely accord with the 7 earlier instances detailed. Thus, no other than the monad organism being present, again its flagellated stage was first detected two or three days after cessation of pyrexia both spontaneous and induced; and more clearly than before the liberated flagellar filaments have been seen, knobbed or knotted, as originally described by Laveran: the phagocyte rapacity was also common, if not always manifested so eagerly as described above; and as regards the characteristic pigmentation, it has seemed more likely that the large black particles within leucocytes are derived from involuted organisms. I have again observed a tendency of the spheroid-bodies to subside during the height and persistence of pyrexia, subsequently to re-appear with crescents, if quinine have not been administered. Renewed attention being paid to the asserted diagnostic import of certain blood-disc changes (*vide Indian Medical Gazette*, January 1886) in remittents and typho-malarial fever, it has still appeared to me here that the intra-cellular aspects in question are neither constant nor uniform enough to be solely relied on as a sure diagnostic means; whilst all forms of pigmented bodies have seemed to be pathognomonic. An example of marked urobilinuria, concurrent with melanæmia and the monad-infection of ague, is detailed below.

CASE 8.—*Ague* and *Urobilinuria,* concurrent with *Melanæmia* and monad *Blood-organisms.*—Patient, a stalwart Hindu, æt. 30, labourer, was admitted on 25th November 1887, immediately after arrival at Bombay from a neighbouring marshy district, where for a month he had been engaged in cutting fresh grass; and owing, he said, to the exposure to sun, hard labour, and scanty food, had become ill with fever about a week before resorting to hospital: no medicine taken. On evening of admission, t. 103·4° (rising to 104·2°) p. 108, : r. 40; there was general distress, vomiting of bile and burning sensation in the epigastrium and splenic region: no hepatic or splenic enlargement, and no gastric tenderness. Rigors preceded and sweating followed the febrile paroxysm; t. on the following morning 97° F. Under salines and diaphoretics, four succeeding smart daily paroxysms were watched; maximum axillary temperatures of 105° being noted at second and third paroxysms; and at the fourth being still 104°. Quinine in two 10-grain doses was given on the 30th November, which did not, however, check the fever, another paroxysm (m. t. 104°) ensuing. On December 1st, therefore, m. t. 98°, three 10-grain doses of quinine were administered, after the last of which the t. did not surpass 99·8°; during the next three days the body-heat remained at 97°—98°, and for the eleven days following it never rose beyond 98·4°. Not more than 50 grains of quinine were given, nor any other antiperiodic drugs; and with good food the patient made a gradual and continuous recovery, only slowly, however, regaining weight. Without the signs of jaundice, this man was noticed as having a peculiar bronzed complexion, which seemed on his rallying to subside too soon for mere solar darkening of the skin.

The urine was copious, assuming a deep blood-like hue when passed at night during the febrile state, and soon decomposing; whilst during the non-febrile intervals it presented simply a high colour and remained tolerably clear. As samples, the night's urine of the 27th measured 35 ounces, had a deep-red coloured hue, and by early morning had become ammoniacal, with (as described), a copious, flocculent, somewhat 'sticky' sediment; sp. gr. 1017, giving the re-action of bile-pigment, and with heat and acid a cloudiness only indicative of albumen; the chlorides $\frac{1}{3}$ volume of liquid, of sugar no trace: whilst the fresh

morning urine was of clear amber hue, faintly acid re-action, sp. gr. 1016, and with slighter traces of albumen. Microscopic examination of the night-urine showed the defined fawn-tinted sediment to consist of pale granular matter, a few epithelial tube-casts, phosphate-prisms, without any visible traces of red blood-discs: the supernatant fluid being also free of clot or red corpuscles, of a deep and clear 'lake' hue, notmuch changed on the addition of ammonia-solution, and not containing more than $\frac{1}{20}$ volume of albumen on coagulation by heat, which also did not destroy the red hue. Nor did the chemical tests for blood, of guaiacum and ozonic ether, here give very positive results. And, further, a scrutiny of the same night-urine with the microspectroscope, made by Dr. Deshmook, revealed only a "dark band in the green, not an absorption-band of blood-colouring matter but probably of urobilin." [Control inspection of urine, containing blood with small clots, showed in all respects a manifest difference to these specimens, in the positive and characteristic re-actions obtained.] Consentaneously with arrest of fever the dark aspect of the night's urine at once changed to a clear sherry-wine tint, and its sp. gr. reduced to 1007; two days later it was still deeper than that of the day's urine, albumen and bile-pigment being also indicated; and after two other days the night-urine was of nearly normal hue, sp. gr. 1005, albumen absent, lithates present: subsequently the urine became even pallid, for a time only. It was stated that never before the present attack, and not until seven days previously when fever came on, had the urine presented a similar bloody appearance; at night only had this been noticed, the day's urine being nearly natural: no particular uneasiness in the loins was ever complained of. The bowels were rather costive: appetite became considerable when the fever left: body weight was lowest (127 lbs), and pulse slowest (60) on fifth day after defervescence, convalescence thence beginning; no local complication was observed, nor any hæmorrhages.

Microscopical examination of the blood was commenced on the patient's admission: the specimens of blood seemed thin and pinkish. Many leucocytes and larger pale nucleated cells were seen, several of which were dotted and some quite loaded with black pigment-granules and clumps; pallid red-discs being sometimes present in the large spleen-cells, along with the pigment masses, whose hue varied from brown to black: with defervescence these melanæmic aspects quickly disappeared. At first, small specks of pigmented plasmic matter were present, and free dark granules in coccus-like groups. The red corpuscles were commonly thin, pale, and shrunken; sometimes vacuolated, without however offering a veritable *plasmode* aspect, either then or afterwards. Specimens taken, during rigor and high fever, showed a few hours later incipient crystallisation in the form of clear reddish rhomboid plates, directly springing from some red discs, thereby partially effaced, the larger plates appearing quite free. Indications of free pigmented organisms were scanty, these consisting of spherules occasionally tending to crescentic shape; and on the third day after exhibition of quinine with defervescence, I detected a distinct large spheroid in which the pigment-grains were dispersed and actively moving, as occurs at the stage of flagellation; vibratory thrills around not, however, being seen, probably from delay of search: subsequently nothing more peculiar was noticed.

*Remarks.*—This Case concurs as regards the blood-state with Cases 6 and 7 detailed above at page 144; yet, whilst the melanæmia was not deeper, and the monad organisms not so numerous, the urine displayed a hæmatinic aspect undistinguishable at a glance from that of hæmoglobinuria, as occasionally seen in remittent fever cases here. The limitation, too, of such blood-like appearance to the febrile periods, seems significant of metabolic changes attendant on the morphological, which microscopically were mainly referable to the spleen—this organ being also, if not much enlarged or tender, the chief seat of a persistent

burning sensation during the acuter stages of illness. Further, assuming that the deep-red aspect of the urine was due to an abundance of urobilin pigment, it may be noted that urobilin can be formed from bilirubin, and that bilirubin is now generally supposed to be formed from hæmoglobin ; such transformation being, however, commonly thought to take place within the liver, which did not appear so much implicated as the spleen in this case. A small quantity of urobilin being present in normal urine, urobilinuria has been regarded as only the exaggeration of a physiological phenomenon ; and indicative of insufficiency or, if prolonged, of disorganisation of the hepatic cells. Doubtless hepatic derangement ensues on the malarial infection ; yet secondarily, I should say, to the splenic ; and I would connect, in this instance at least, the state of the urine with that of the blood. Admittedly, the pigmented organisms were here so few that they might have been overlooked, but they were present : and the malarial nature of the blood and urine-changes which prevailed, was, I may add, collaterally shown by the fact of the patient's wife, who worked in the fields with him, displaying in hospital marked paroxysmal fever. Thereupon her blood was examined and found to contain unmistakeable parasites, both sessile on the red discs and free as spheroids and crescents ; the phagocyte phenomenon being as well evident. The liver and spleen were even much more considerably enlarged, yet the urine was merely high-coloured and did not contain any albumen : the specific fever was checked by quinine in her, as readily as in her husband's case.

Summarising the whole data, I consider there is proof enough that the genuine "Ague-state"—the '"malaria-process'" in older term and in new the "malaria-infection"—is pathologically distinguished by a visible living blood-contamination (*a*), having hæmatozoic rather than hæmatophytal affinities (*b*), and displaying a relationship to clinical symptoms which, if often less precise than obtains with pathogenic bacterial infections, may none the less be real (*c*). Further, it has been shown that in arresting malarial pyrexia, the drug quinine does not with equal promptitude annihilate the blood-parasite (*d*) ; this datum also indicating a probable different relationship of phenomena, in the monadic as compared with bacteric infection. That the hæmatozoa undoubtedly present in ague, holds a close, if not causal, relation to that disease, may be inferred from first their constituting an adequate pathogenic influence, and next their exclusive limitation to this one morbid affection : nor need such influence be annulled by seemingly conflicting evidence regarding the details of association. According to my observations, the visible blood-contamination may be more constant and uniform than concurrent pyrexial phenomena; and hence the inference that it is fundamental, whilst 'fever' is rather a contingent event. Certainly not all fever in malarious subjects is necessarily monad-pyrexia ; and by experience I have been led to recognise at least three forms of such 'fever'—namely, first the genuine specific form, with its positive blood-aspects ; next and oftener in old cases, the consecutive residual or quasi-reactive form with its negative blood-state, which may have simulated the genuine type in a clinical sense ; and, lastly, the pyrexia pertaining to a superadded infection, which for a time supersedes the monadic—as, for example, was demonstrated for enteric fever by Dr. Laveran (*l. c.*, p. 378). As to nature and causation, I would add that present results serve to explain the paroxysmal and periodic character of paludal fever, through the corresponding definite duration and reproduction of a living contagium. That such pathogenic agent should be zooic rather than phytal, is a datum of etiological import ; because Infusorial life is known to prevail under different and more restricted condition than the Bacterial, and hereby a clue may be gained as to the sources of ague-infection. The foreign and nondescript term of "Malaria" adopted in Britain since 1827, may soon have to be abandoned

if not in favour of the prior indigenous name of "Marsh" poison, at least of a designation referring to definite conditions of soil, moisture, and water-supply. Not long since, the late sagacious Professor of Military Medicine at Netley wrote respecting the new researches in Europe, as follows: 'Should future investigations by independent observers in other malarious regions confirm these conclusions, it would be difficult to over-rate their importance' (Maclean in QUAINE'S DICTIONARY, Part II, p. 914); and now it may be seen how far, as regards India, such confirmation has been realised.

Government of India Central Printing Office.—No. 1 S. C.—5-3-88.—270.

# Description of Plate.

PLATE I.—SCHIZOMYCETES, MYCOIDEA, AND NYMPHÆA BLIGHT.

Fig. 1. Organisms in the blood of a horse ×680.

" 2. Spirillar variety of choleraic comma-bacilli ×1270.

" 3. Blighted leaf of *Nymphæa stellata*. Nat. size.

" 4. Vertical section through a portion of a leaf of *Cinnamomum iners* affected by Mycoidea ×190.

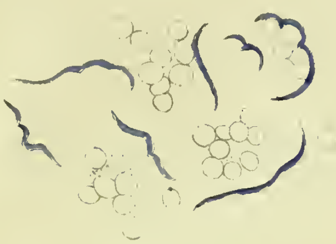
" 5. Leaf of *Cinnamomum iners* viewed from above, showing a patch affected by Mycoidea. Nat. size.

" 6. Same leaf from below. Nat. size.

" 7. Vertical section through a patch affected by Mycoidea, showing algal cells extending from the superior, subepidermal thallus through the greater part of the thickness of the leaf ×360.

" 8. Portion of the same section, showing superior subepidermal thallus and an emergent filament ×360.

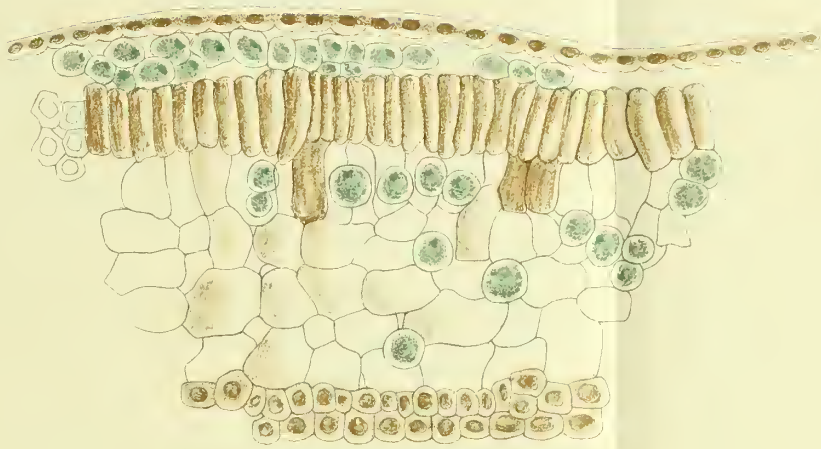
" 9. Portion of the same section showing a mass of algal cells projecting deeply into the tissue of the under-surface of the leaf and giving off emergent filaments ×360.


1. × 680


2 × 1270.


3. nat. si...


6. nat. size.


4 × 190.


5. nat. size


7 × 360.


8. × 360.


9. × 36...

Lithographed at the Survey of India Offices, Calcutta, May 1887.

# Description of Plate.

## PLATE II.—A NEW GENUS BELONGING TO THE FAMILY USTILAGINEÆ.

Fig. 1. Vertical section of a leaf of *Nymphæa stellata* showing spores in substomatic spaces ×360.

,, 2. Section through the entire thickness of a leaf showing distribution of spores ×119.

,, 3. Section through the upper part of a healthy leaf, showing large substomatic space ×360.

,, 4. Fine mycelium giving origin to thicker fertile filaments bearing young spores ×680.

,, 5. Vertical section through entire thickness of a healthy leaf of *Nymphæa stellata* showing subepidermal spaces, great spaces over the inferior epidermis and idioblasts ×119.

,, 6. Fine mycelial filaments with simple haustoria and thicker sporiferous hyphæ.

,, 7. Head of a promycelial filament with sporidiiferous cells.

,, 8. Germinating spore still connected with the Mycelium ×970.

,, 9. Spore with promycelium.

,, 10. Sporiferous filaments and spores in various stages of development ×970.

,, 11. Conjugating pair of sporidia ×970.

,, 12. Sporidia bearing secondary sporidia, and still attached to the promycelium.

,, 13. Spore, promycelium, and conjugating sporidia.

,, 14. Conjugating sporidia.

,, 15. Portion of epidermis with promycelia emerging from the stomata ×680.

,, 16. Terminal portions of promycelium showing septation and sterigmata.

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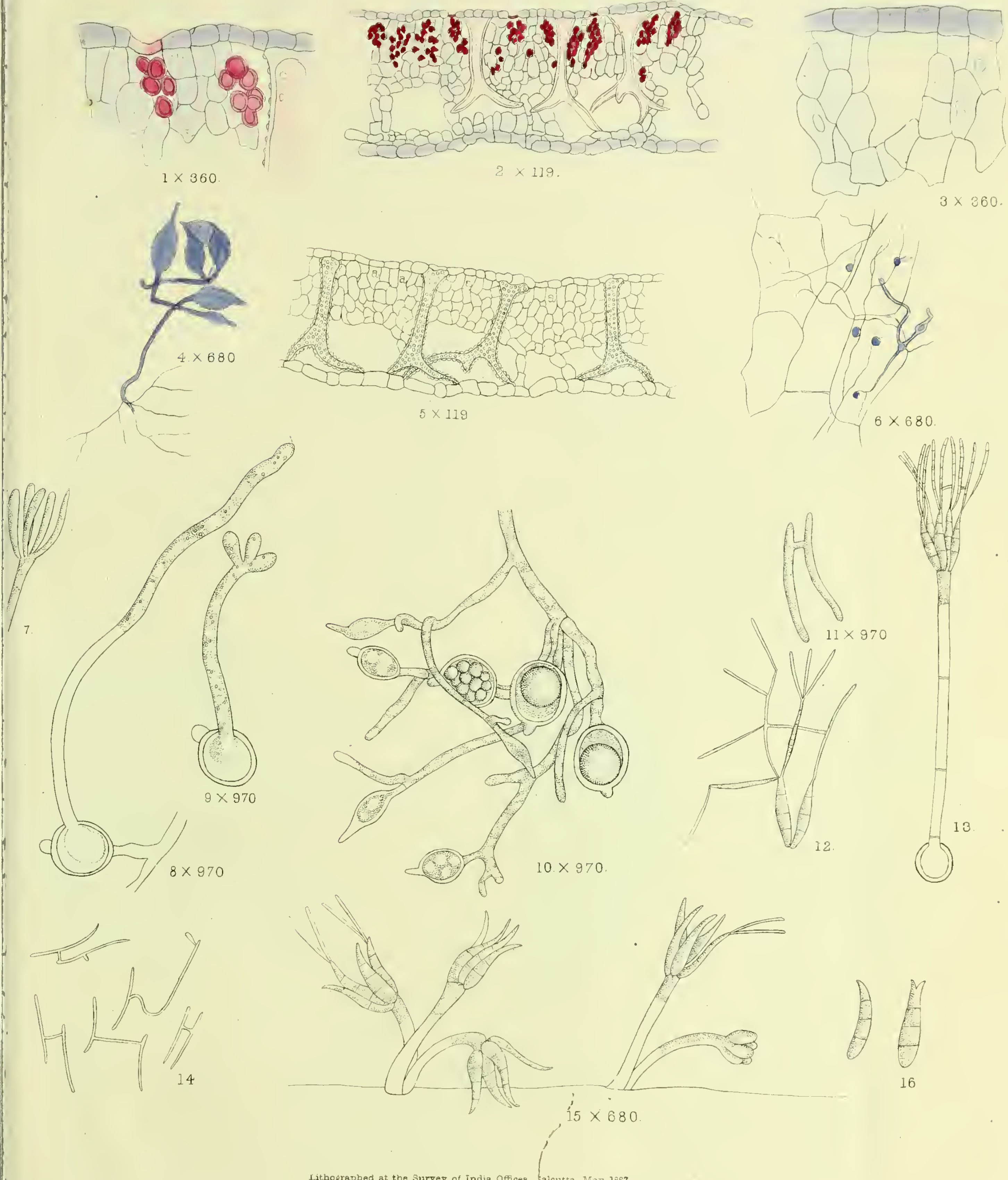


Lithographed at the Survey of India Offices, Calcutta, May 1887.

## Description of Plate.

PLATE III.—A NEW GENUS OF ENTOPHYTIC ALGÆ.

Fig. 1. A leaf of *Limnanthemum indicum* affected by the alga. Nat. size

" 2. Portion of one of the algal patches × 190.

" 3. Two young algal cells seen through the epidermis × 360.

" 4. Vertical section through the upper part of a normal leaf × 360.

" 5. Vertical section through the upper part of a leaf containing algal cells × 360

" 6. Portion of subepidermal palisade tissue containing *two* resting algal cells × 360

" 7. Free resting cell from sediment of *water* containing affected leaves × 360

" 8. Young zoosporangium *with* multinucleate protoplasm × 680.

" 9. Free zoospore × 970.

" 10. Zoosporangium *with* retained zoospores × 680.

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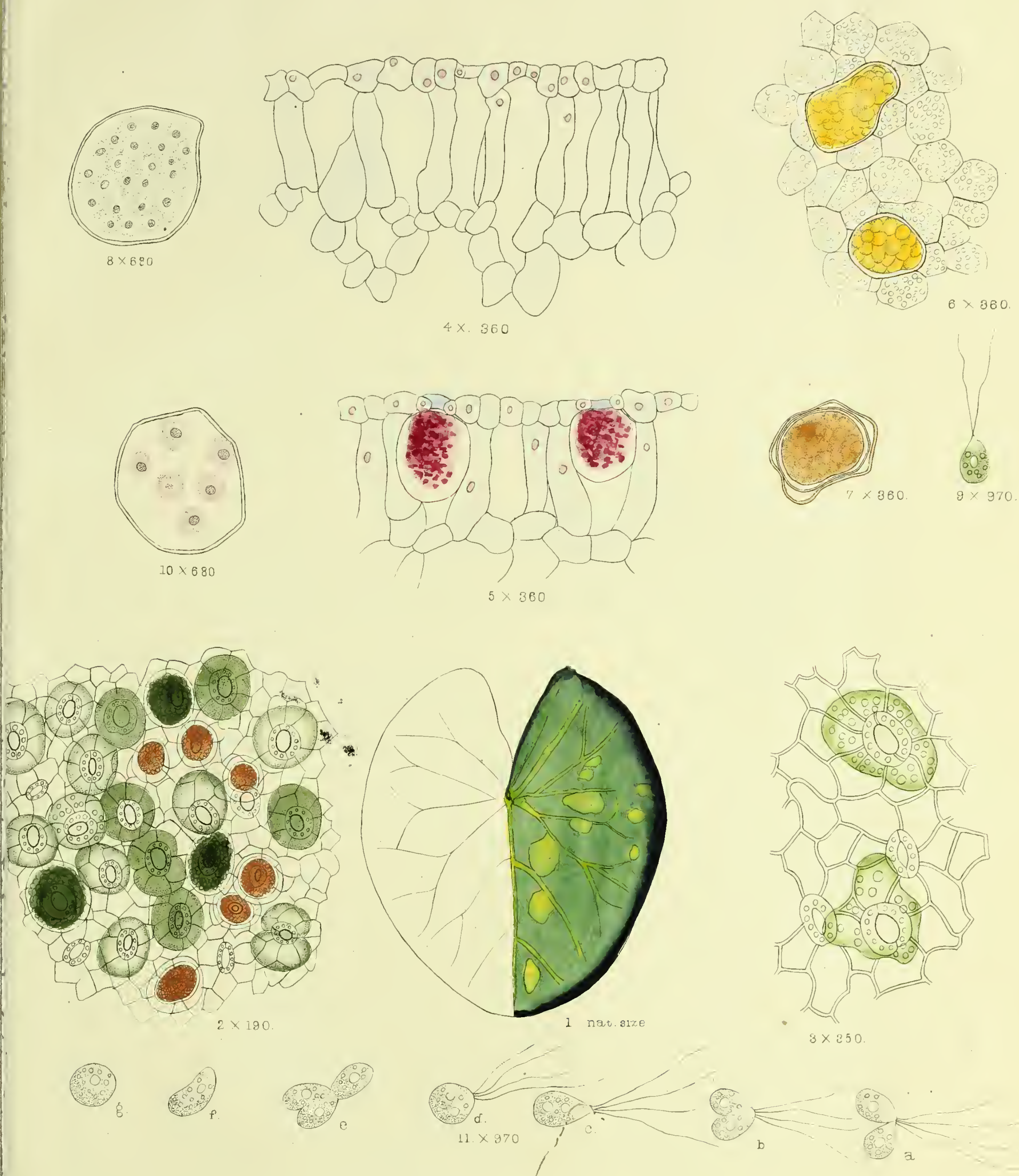
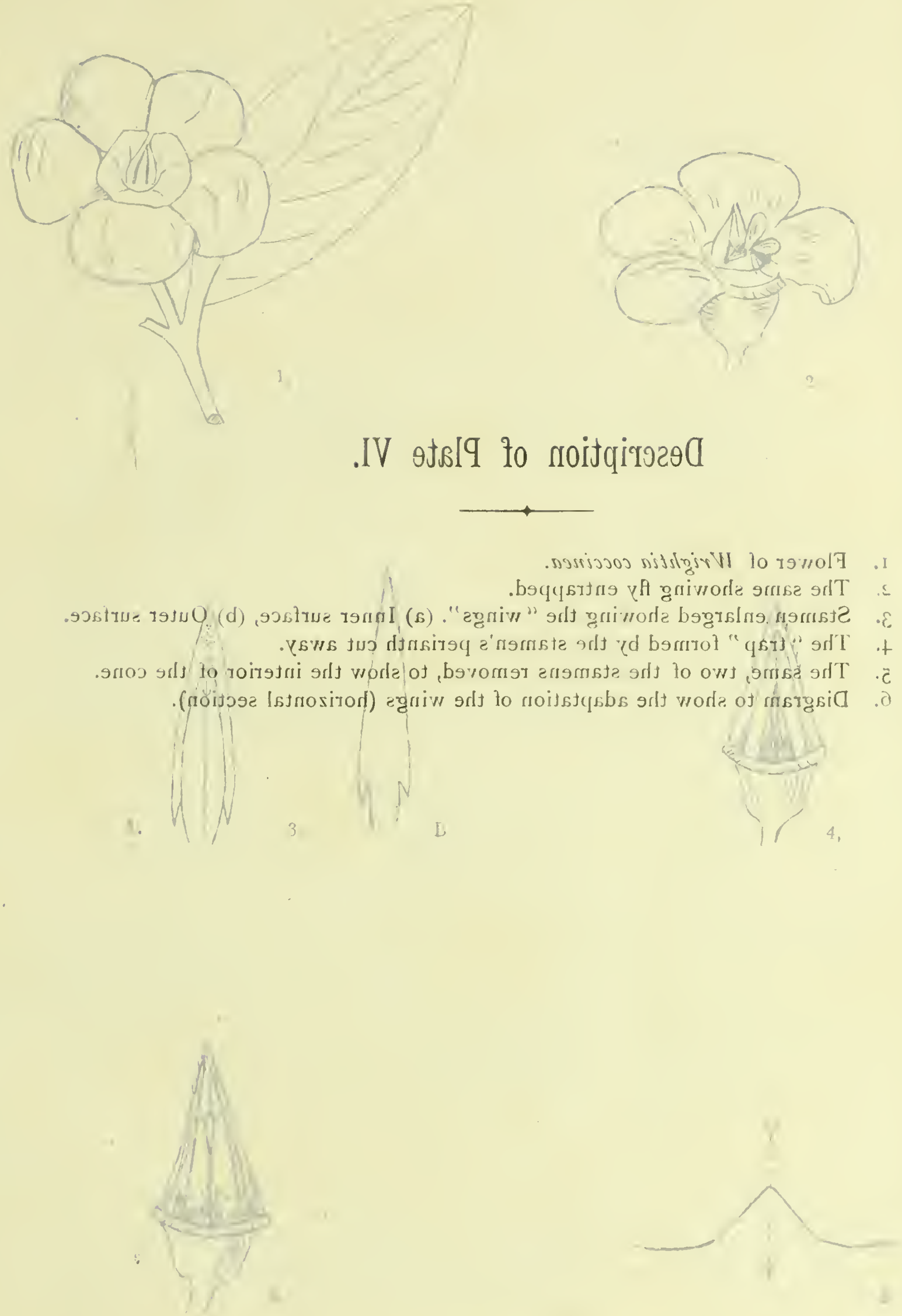
„ 10. Zoosporangium with retained zoospores × 680.

„ 11. Conjugation of zoospores × 970.



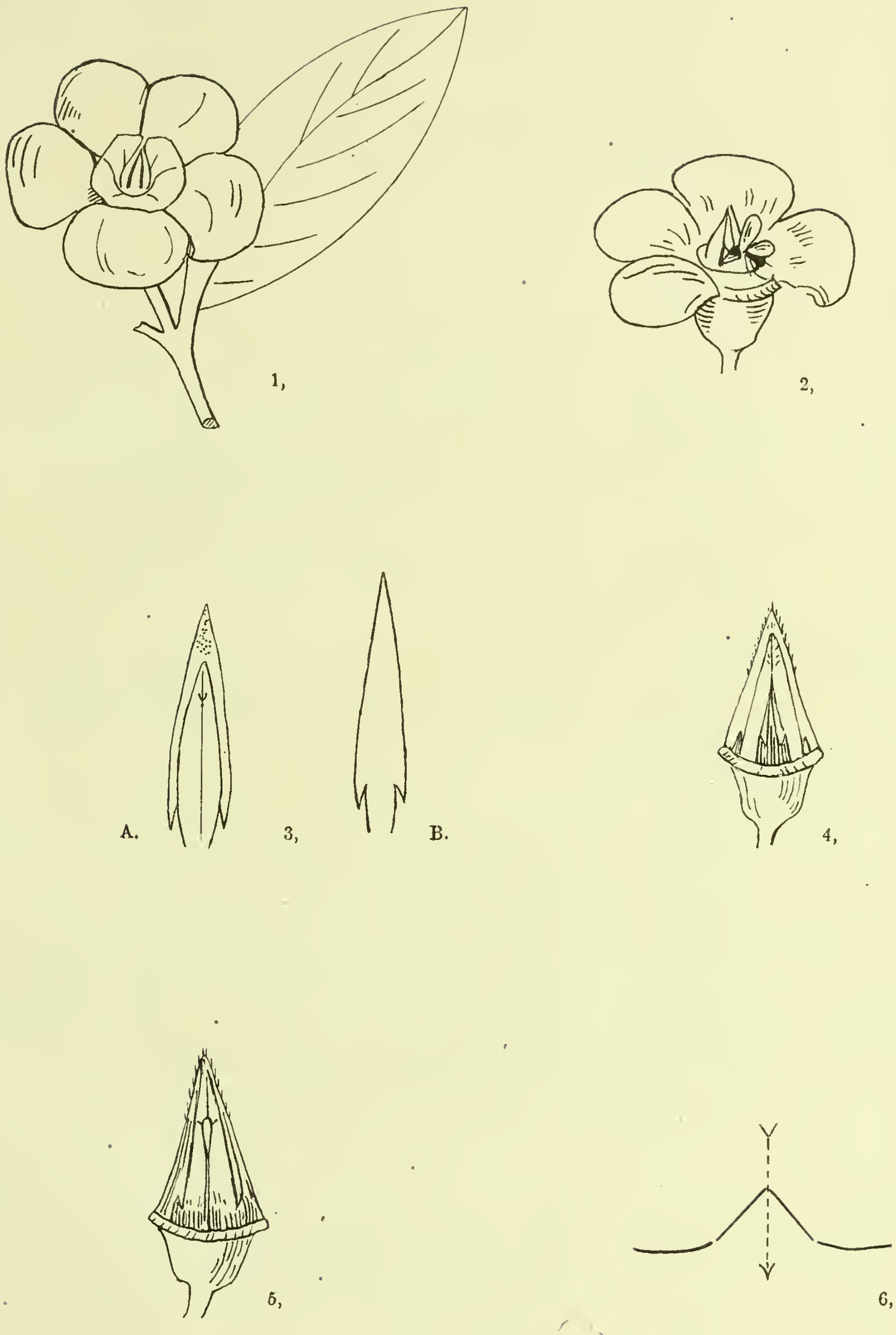

Lithographed at the Survey of India Offices, Calcutta, May 1897.




THE FLY CATCHING APPARATUS OF WRIGHTIA COCCINEA.

# Description of Plate VI.

FIG. 1. Flower of *Wrightia coccinea.*
,, 2. The same showing fly entrapped.
,, 3. Stamen enlarged showing the "wings". (a) Inner surface, (b) Outer surface.
,, 4. The "trap" formed by the stamen's perianth cut away.
,, 5. The same, two of the stamens removed, to show the interior of the cone.
,, 6. Diagram to show the adaptation of the wings (horizontal section).




THE FLY CATCHING-APPARATUS OF WRIGHTIA COCCINEA.

Printed at the Lithographic Office, Survey of India Department, Calcutta, July 1887.

# Description of the Illustrations.

## PLATE IV.

FIGURES 1 AND 2. Spirillum of the rat × 800 diameters: Temperature of the rat 101·6° F.

FIG. 1.—Fresh blood on watching; temperature of the rat 103°F. "The organisms have the aspect of veritable spirilla, moving actively amongst the fibrine bands and in clear serum spaces, yet not easily seen even with the $\frac{1}{16}$ inch immersion." (*Extract from Note-book.*) A white corpuscle is shown to the right above; below at * are some dried and stained organisms showing a tendency to divide, and one with a central dark spot.

„ 2.—Blood after 20 hours, inclosed in sealed cell. "In open spaces are seen 2—4 spirilla in active constant movement (chiefly rotation), quite distinct from the languid and intermittent movement of the bacteria which are also present." (*from Note-book*). (*a*) Micrococci, (*b*) Bacillar rods, (*c*) The spirillar organisms.

CHART NO. 1.—Bi-daily observations of temperature and blood-state of the rat showing the above spirillar contamination. *Memo.*—When spirillar organisms "doubtfully seen," then other minute bodies detected, often having the aspect of such organisms either immature or else disintegrating.

„ NO. 2.—A sample of ordinary temperature ranges in a rat having healthy-looking blood.

„ NO. 3.—A sample of temperature range in a newly-captured rat having on the first three days monads in the blood, and subsequently suffering from swelling of the feet and paraplegic symptoms.

„ NO. 4.—The entire temperature and blood data of the experimental monkey, into whose blood the rat's monads had found their way after intra-peritoneal injection. Day 1, is the evening of experiment; on day 2, there are two positive observations, and two also on day 3, after which no more monads visible. The "uneasiness" of the monkey on the first 3 or 4 days was not more than accountable by the mild irritation of puncture, and the temperature-range seemed hardly disturbed.

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FIG. 1.—Fresh blood on watching; temperature of the rat 103°F. "The organisms have the aspect of veritable spirilla, moving actively amongst the fibrine bands and in clear serum spaces, yet not easily seen even with the $\frac{1}{10}$ inch immersion." (*Extract from Note-book.*) A white corpuscle is shown to the right above; below at * are some dried and stained organisms showing a tendency to divide, and one with a central dark spot.

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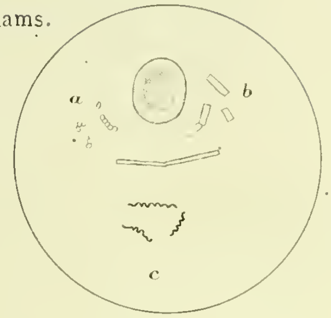
## Spirillum of the Rat.

Fig. 1. Fig. 2.

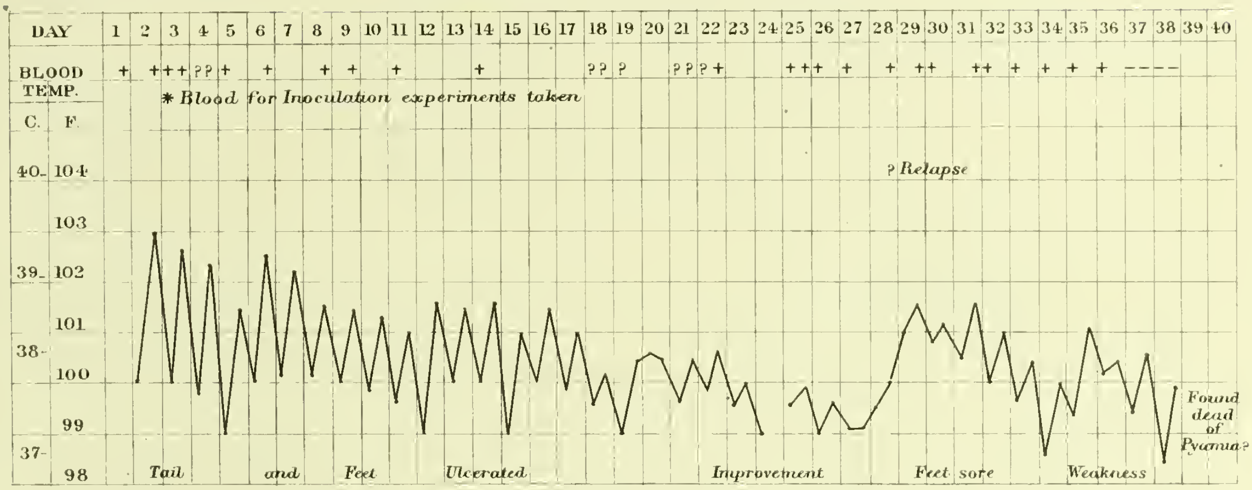
× 800 diams.




## Chart No. I.

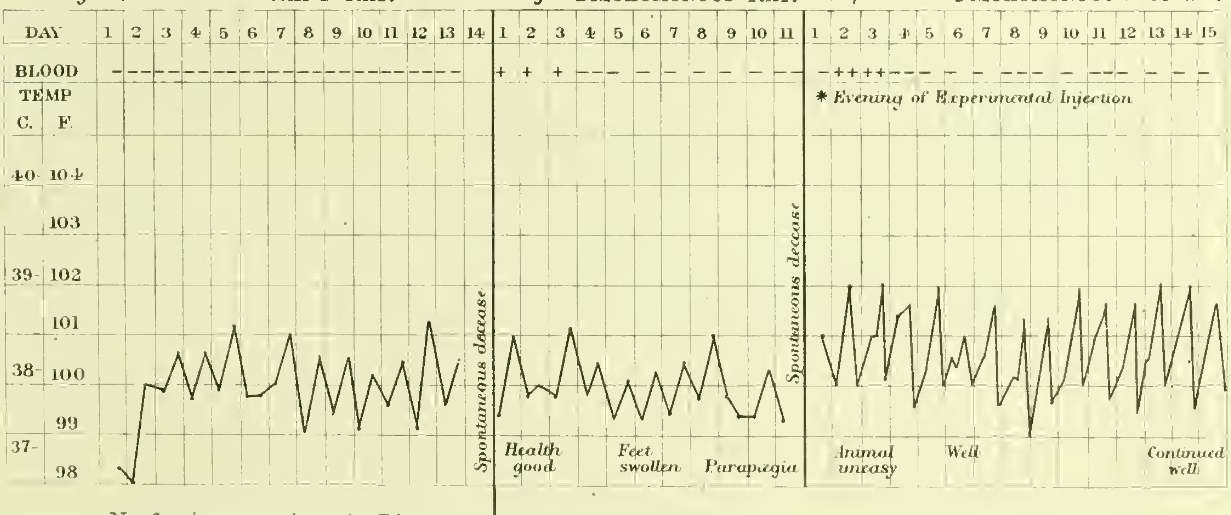
Spirillar Rat.

*August-September 1885.* *Daily observations of Temperature and Blood-state.*




Memo. on Blood. + Spirillum present. ? Sp. doubtfully seen. —Sp. absent, on morning or evening of observation days; beginning with day of capture, when first scrutiny made.

**No. 2.** *July* Healthy-Looking Rat. **No. 3.** *July* Trichomonous Rat. **No. 4.** *September* Trichomonous Monkey.




No foreign organisms in Blood. + Trichomonas present. — T. not visible in Blood.

Lithographed at the Survey of India Offices, Calcutta, December 1887.

# Description of the Illustrations.

## PLATE V.

Figures 1 to 4 form a series, all magnified 500 diameters: Figures 1 and 2 are hæmatophytes, and figures 3 and 4 are hæmatozoa, as described in the text.

The blood-disc introduced in each figure will serve to aid intercomparison of the organisms.

FIGURE 1.—Bacillus anthracis (copied from Ziegler).

FIGURE 2.—Spirillum (Spirochæta) Obermeieri, aspects seen in blood during life; straight and unfolded, also clustering and adhesion to red-discs (human).

" 3.—Tricho-monas sanguinis (rat) common aspects, when semi-quiescent.

" 4.—Filaria sanguinis hominis (Lewis).

FIGURES 5 and 6.—Flagellated organisms from the rat's blood.

" 5.—× 1000 diameters: (*a*) from osmic acid preparations in which the unilateral membrane is clearly indicated and a bright speck near the tail ends. The blood-disc gives relative dimensions. (*b*) from aniline-stained preparations, in which the lighter stained edge corresponds to the same lateral membrane; the flagellum is not wholly tinted (dated Feb. 1885).

" 6.— × 800 diameters. Changes undergone by rat-monads in fresh blood diluted with 75 per cent. solution of common salt in water, hermetically sealed and kept at 70° to 80°F. For description see the text: (*a*) from a series of trials made in February 1885; (*b*) from other experiments made in September following, some slight difference being apparent.

FIGURES 7 and 8.—Organisms from the blood of diseased animals affected with (surra).

" 7.—× 1000 diameters. From aniline-stained preparations: to the right are four of the "monads" with two red discs (one shrivelled) and a larger nucleated white cell; to the left are some smaller plasmic (?) particles* × 1200 diameters as seen with $\frac{1}{12}$ inch oil-immersion lens of Powell and Lealand, London, in February 1885, and then designated as possibly incipient flagellated organisms.

* They were sometimes found radiating round red-discs, or joined end to end, so as to appear of great length, when their ends may unite; or joining laterally so as to seem thickened, doubled, or branching; or clustering at various angles so as to form meshes or clumps; or intermingled variously. Below is seen the attachment of the monads to red-discs, so striking in the fresh state.

FIGURE 8.—× 800 diameters. Organisms from inoculated animals, as named in the figure. The differences apparent amongst the monads of mule, dog, and monkey did not seem at all considerable, constant, or characteristic; and it was considered that they hardly, if at all, surpassed a natural variation, easily surmised and understood.

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Fig. I.
BACILLUS ANTHRACIS.

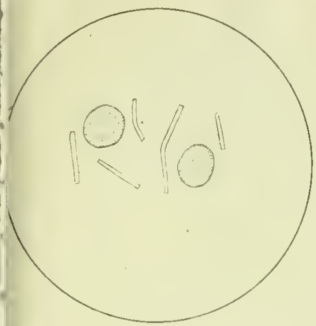


Fig. 2.
SPIROCHŒTA OBERMEIERI.

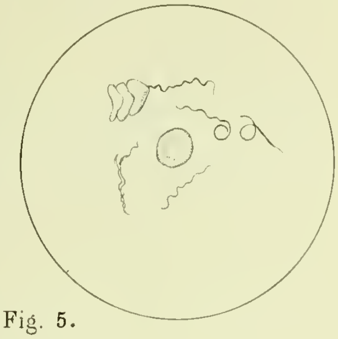


Fig. 3.
TRICHOMONAS SANGUINIS.

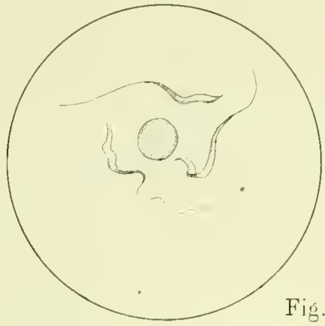


Fig. 4.
FILARIA SANGUINIS HOMINIS

Fig. 5.
ORGANISMS OF THE RAT.

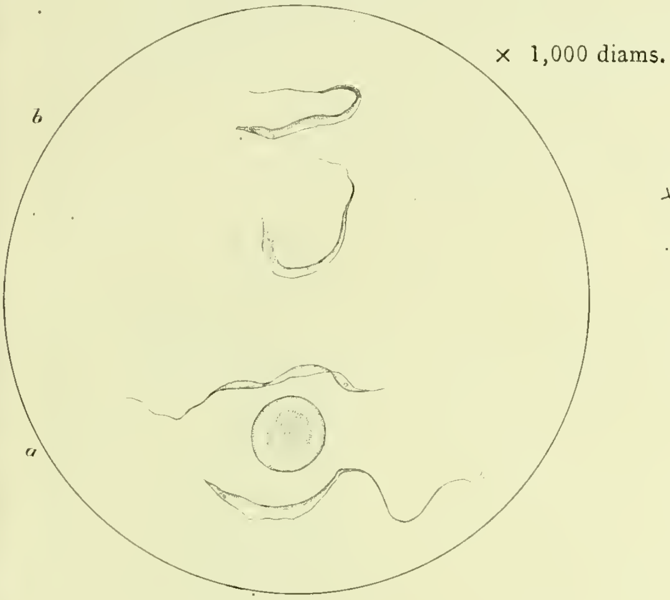



Fig. 7.
ORGANISMS OF SURRA (Mule).

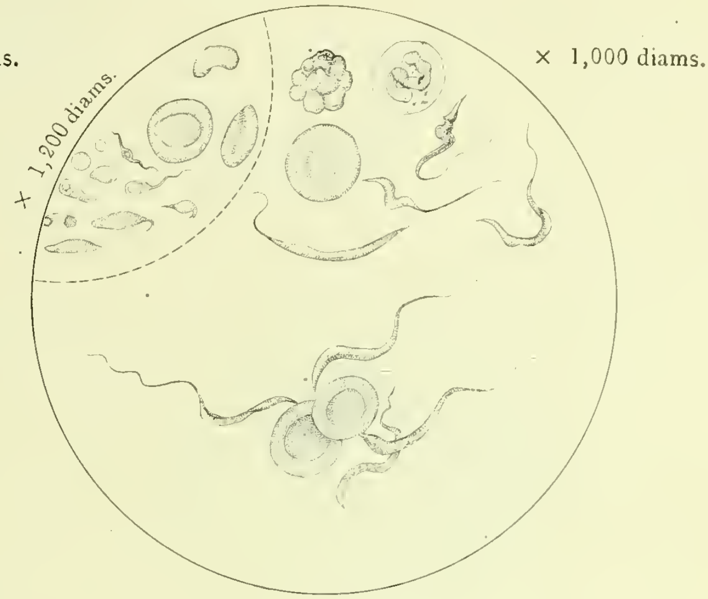



Fig. 6.

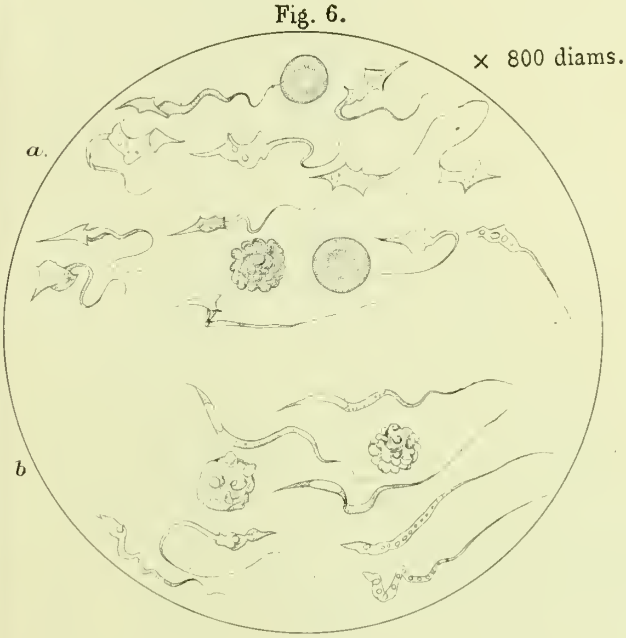
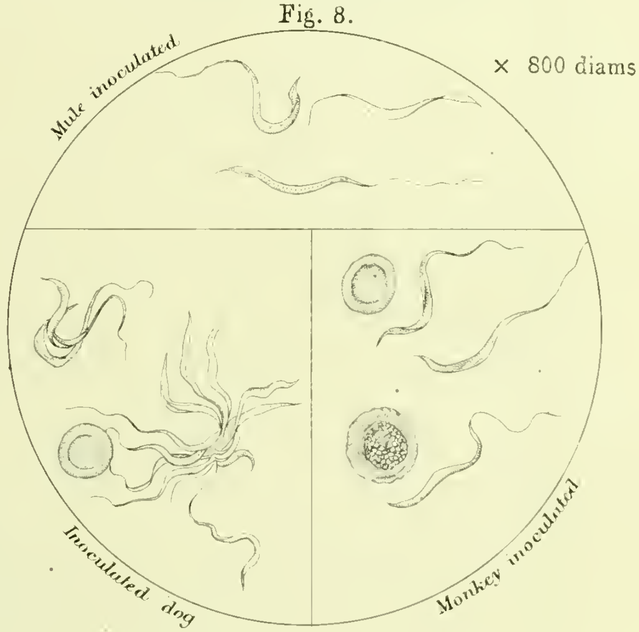



Fig. 8.




Lithographed at the Survey of India Offices, Calcutta, December 1887.

## Description of Plate VII.

FIG. 1.—Large comma bacilli in the adenoid tissue and Licberkuhn's crypts, and also below the muscularis mucosa, of the ileum. Case 4.

„ 2.—Small comma bacilli in the interior of Licberkuhn's crypts and extending into the space between the bases of two villi in the ileum of Case 5.

„ 3.—Group of bacilli from the space between the villi in Case 5, more magnified than in Fig. 2.

„ 4.—Another group from the deeper part of the cavity of the crypt.

„ 5.—Spirilla in a Licberkuhn's crypt (transversely divided) from the vermiform appendix of Case 9.

„ 6.—Filiform bacilli in the substance of the colon of Case 5.

Fig 1×400

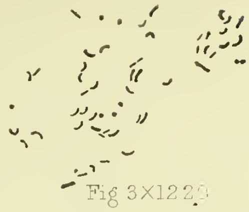


Fig 3×1220

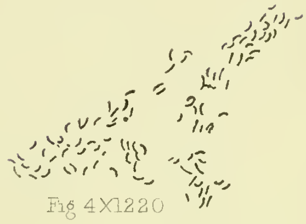


Fig 4×1220

Fig 5×550

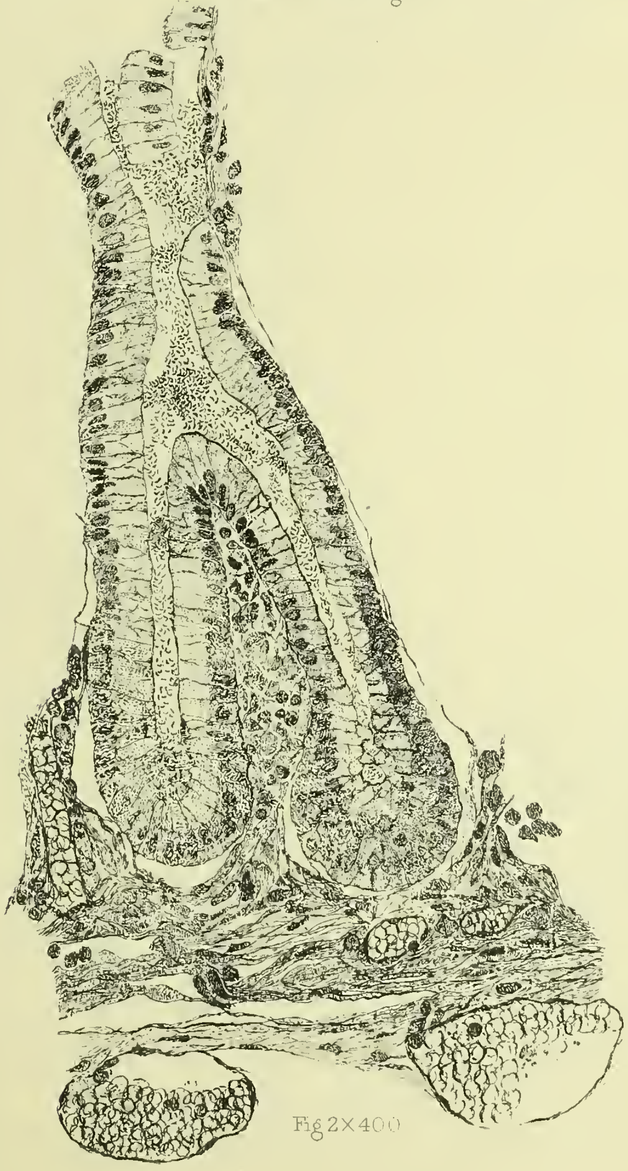


Fig 2×400

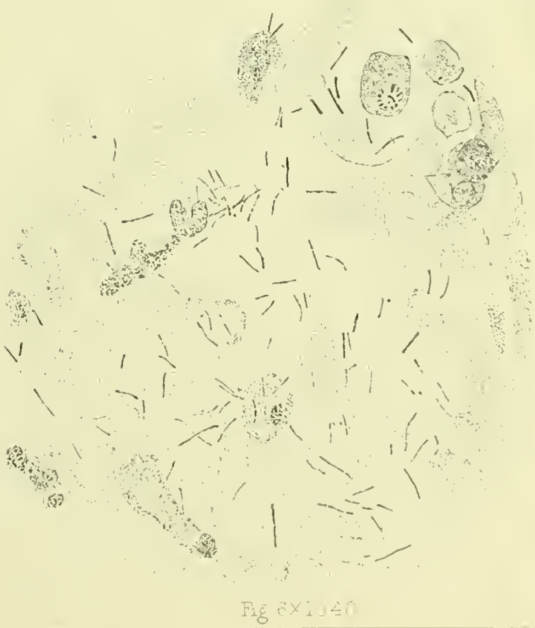


Fig 6×1040

Photo-etching from the original pen and ink drawings - Survey of India Offices, Calcutta, September 887

# Description of Plate VIII.

FIG. 7.—Micrococci within and without a small blood vessel from a purulent focus in the lung of Case 5.

„ 8.—Micrococci amongst the blood corpuscles in a vessel of the kidney of Case 1.

„ 9.—Spotted bacilli infiltrating the mucous membrane surrounding an ulcer of the stomach in Case 8.

„ 10.—The same bacilli more magnified.

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Fig 7 × 500

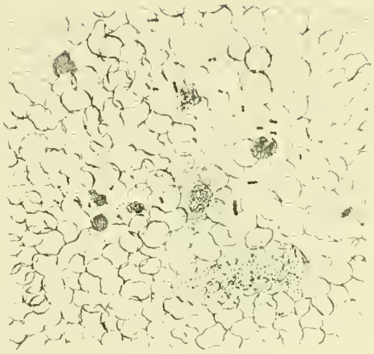

Fig 8 × 500

Fig 10 × 1220

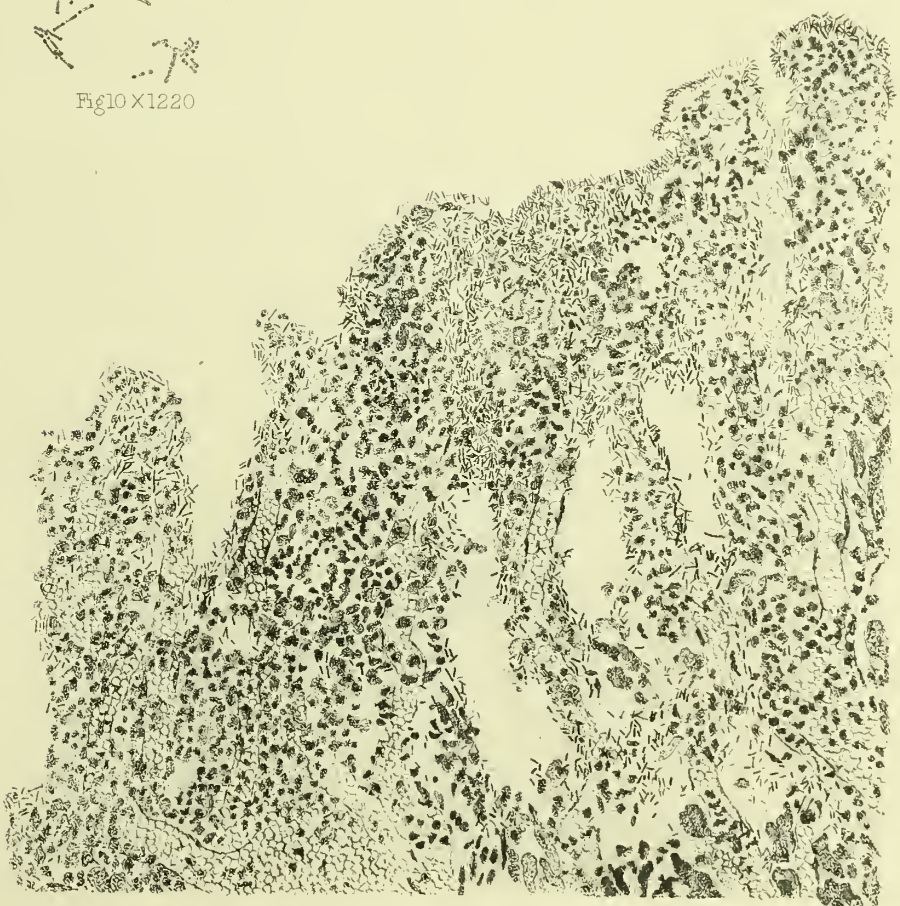

Fig 9 × 250

G. Bomford, del.

Photo-etching From the original pen and ink drawings – Survey of India Offices Calcutta September 1887.

FIG. 13. A group of *red blood-discs* agitated and distorted by a slender lashing filament, which had become detached from a neighbouring flagellated body, during its brief movements under scrutiny: the thrilling aspect amongst the corpuscles was very evanescent, and being detected in two or three separate parts of the field, it seemed that more than one flagellum was liberated or else after detachment one had continued to travel. An appearance not very rare.

FIG. 14. A *phagocyte* containing in its interior two pigmented spheroids.

FIG. 15. *Melanæmia.* A group of pigmented structures seen together in the blood, the day after 'crisis' of paroxysmal fever—CASE NO. 6. At *a* an enormous nucleated cell (? splenic endothelium) loaded with pigment masses ('melanine', so-called), extra-nuclear in site and opaque-black in tint: many such cells were present. At *b* an ordinary smaller leucocyte; also common. At *c* spheroidal, cylindrical, and semi-lunar organisms (some red corpuscles also added), which were seen in proximity and likewise not infrequent.

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an evacuated Crescent, with the pigmented spheroid body, which had thence become extruded; fresh specimen non-febrile; and numerous free organisms also present, including the flagellate.

*b.—The Spheroidal. Bare.*

FIG. 6. Pigmented *spherules* seen in fresh blood, during the stage of 'rigor'—Quartan ague.—CASE NO. 1. *a. a.* Unaltered red discs. *b.* A spheroidal body, at successive periods of 20 minutes watched. At * below, a pigmented body with large nucleus, and a red-corpuscle with spheroid apposed, observed in the same specimen: see also series A, figure 3, above.

FIG. 7. A group of four *spherules*, from the same case, and taken at estimated 'acme' of the febrile paroxysm (t. 103·4°F.) Below, three other spherules taken four hours after cessation (spontaneous) of pyrexia. To show amœboid and varying aspect of the spheres, and of their pigment contents.

FIG. 8. Other *spheroids* taken on day of expected relapse, before the setting in of 'rigors': magnified 1,000 diameters. Shows a tendency to partition of Spherules, and to assumption of Crescent shape?

FIG. 9. Other *spheroids* taken at 'hot' stage of a paroxysm (t. 106°F.): showing an apparent division or disruption of some bodies, with dispersion of the dotted granules (? germs) thence resulting. CASE 1: quinine not given. At * a similar aspect of spontaneous partition, with separation of the pigment, noted in CASE No. 2, at non-febrile state (quinine administered).

FIG. 10. *Pigmented plasmoid particles* frequently seen in the free state, and seemingly indicative of one mode in which free spheroids may be formed afresh; namely, by direct growth of such freed particles.

*Flagellate Organisms*

FIG. 11. A *flagellate or ciliated spherule*, as seen in fresh blood. CASE No. 5, non-febrile and without quinine: the flagella were in very active lashing movement, the central body oscillating slightly, and its pigment-contents also much agitated. Whilst being watched the organism was seized by a wandering, coarse-grained *Amœba*: after a struggle becoming involved; and in the course of 20 minutes carried away, as indicated by *Figures a.* and *b.*: this phenomenon being so frequently witnessed as to appear not merely incidental.

FIG. 12. Another *flagellated body*, from CASE NO. 6, during convalescence on eighth day after arrest of fever, and the blood thenceforth free from visible contamination; quinine exhibited. movements so active that the number of flagella could hardly be surely ascertained: amœbæ here, too, early made their appearance. At * is another pigmented body belonging to a flagellated organism, introduced to show a striated arrangement assumed by the agitated pigment-particles within, during struggles against an invading and finally conquering amœba. At * * three liberated and free-moving *Flagella*, somewhat resembling bacteric spirillar organisms: they are distinctly knobbed midway or at end.

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puscle itself though not pallid seemed a little shrunken, and changed in outline. Within the disc-area, the cocci became dispersed, or variously aggregated, after the manner of black pigment-granules in spherules and crescents. In the liquid medium near, free cocci were present; not arranged in cluster or chain. From Case 6 on fifth day after arrest of fever by quinine and continued exhibition of the drug: axillary t. 98·2° F., Aspect rare. Free organisms were present in the blood.

FIG. 6. A red blood-disc which showed on watching active *amœboid* movements such as here delineated at one instant; and pertaining apparently to a pale, finely granular, plasmic material or body, within the disc-area and clearly differentiated from the coloured portion of the corpuscle. Movements varied and persistent for an hour, at least, without further change; the disc was turned about but not displaced by them. Aspect occasional only. Date the ninth day after arrest of fever by quinine (drug continued: t. 98·6° F. Flagellate organisms seen the day before; no free organisms co-present, and none visible for remaining nineteen days of patient's convalescence in hospital.

FIG. 7. Two red blood-corpuscles, introduced as samples of aspects often visible in healthy blood, as well as the feverish, and evidently attendant on altering physical conditions alone. The separation of coloured and colourless portions of the disc, is as distinct as that following parasitism of the corpuscles, and within slow movements occur; but pigment-formation is absent, and all signs of serial change around.

SERIES B.—FREE FORMS. *A. The Crescentic.*

FIG. 1. Parasitic organisms seen in fresh blood, towards the close of a febrile paroxysm—Quotidian ague—CASE No 2 m.t. 103° F. remarkably little distress shown, and the spleen not projecting and not tender. At *a a.* normal red corpuscles, *b.* a leucocyte; *c. c.* the pigmented *crescents*; at * changes of aspect in a pigment-spot at successive five-minute intervals; and at * * other variations in aspect seen after the sweating-stage: no quinine.

FIG. 2. *Crescents* seemingly held by infolded red corpuscles: aspect not very common. From the same patient, in subsequent apyretic intervals.

FIG. 3. The *three upper* Crescentic bodies are from a specimen (fever stage) of fresh blood, after slight evaporation of the serum; so that the organisms, no longer floating quite upright, are seen partly from their side and their pendant membrane thereby rendered more clearly visible. The *four lower* bodies are from stained and mounted specimens (same patient): at * an unchanged blood-discs; and close by a crescent entire and still enclosed, and in diameter rather less than the disc. Non-febrile stage, and tinted with methyl-violet. The *three lowest* bodies are from febrile specimens stained with fuchsine and violet; they show the membrane either complete or partly shrunken, the organisms often seeming to shrink a little during the process of preparation.

FIG. 4. Some *varieties* in the shapes of fresh Crescents, indicating an approach to the spherical form.

FIG. 5. Three stained *Crescents*, showing irregular distribution of stain and of pigment, after the action of water and of acetic acid. At * apparently

puscle itself though not pallid seemed a little shrunken, and changed its outline. Within the disc-area, the cocci became dispersed, or variously aggregated, after the manner of black pigment-granules in spherules and crescents. In the liquid medium near, free cocci were present ; not arranged in cluster or chain. From Case 6 on fifth day after arrest of fever by quinine and continued exhibition of the drug : axillary t. 98·2° F., Aspect rare. Free organisms were present in the blood.

FIG. 6. A red blood-disc which showed on watching active *amœboid* movements, such as here delineated at one instant ; and pertaining apparently to a pale, finely granular, plasmic material or body, within the disc-area and clearly differentiated from the coloured portion of the corpuscle. Movements varied and persistent for an hour, at least, without further change ; the disc was turned about, but not displaced by them. Aspect occasional only. Date the ninth day after arrest of fever by quinine (drug continued: t. 98·6° F. Flagellate organisms seen the day before ; no free organisms co-present, and none visible for remaining nineteen days of patient's convalescence in hospital.

FIG. 7. Two red blood-corpuscles, introduced as samples of aspects often visible in healthy blood, as well as the feverish, and evidently attendant on altering physical conditions alone. The separation of coloured and colourless portions of the disc, is as distinct as that following parasitism of the corpuscles, and within slow movements occur ; but pigment-formation is absent, and all signs of serial change around.

## SERIES B.—FREE FORMS. *A. The Crescentic.*

FIG. 1. Parasitic organisms seen in fresh blood, towards the close of a febrile paroxysm — Quotidian ague—CASE No. 2 m.t. 103° F. remarkably little distress shown, and the spleen not projecting and not tender. At *a. a.* normal red corpuscles ; *b.* a leucocyte ; *c. c.* the pigmented *crescents* ; at* changes of aspect in a pigment-spot at successive five-minute intervals ; and at * * other variations in aspect seen after the sweating-stage : no quinine.

FIG. 2. *Crescents* seemingly held by infolded red corpuscles : aspect not very common. From the same patient, in subsequent apyretic intervals.

FIG. 3. The *three upper* Crescentic bodies are from a specimen (fever stage) of fresh blood, after slight evaporation of the serum ; so that the organisms, no longer floating quite upright, are seen partly from their side and their pendant membrane thereby rendered more clearly visible. The *four lower* bodies are from stained and mounted specimens (same patient) : at * an unchanged blood-discs.; and close by a crescent entire and still enclosed, and in diameter rather less than the disc. Non-febrile stage, and tinted with methyl-violet. The *three lowest* bodies are from febrile specimens stained with fuchsine and violet ; they show the membrane either complete or partly shrunken, the organisms often seeming to shrink a little during the process of preparation.

FIG. 4. Some *varieties* in the shapes of fresh Crescents, indicating an approach to the spherical form.

FIG. 5. Three stained *Crescents*, showing irregular distribution of stain and of pigment, after the action of water and of acetic acid. At * apparently

# Description of the Illustrations in Plate IX.

## THE BLOOD-ORGANISMS SEEN IN AGUE.

*Magnifying Power—800-1000 Diameters.*

### SERIES A.—INTRA-DISC FORMS.

FIG. 1. Two red blood-discs, showing a minute pale body which at first seemed to be pedunculated (*upper disc*) and then disengaged, when it began to display distinct amæboid movements attended with various marked changes of form (represented at one instant in the *lower disc*), and some increase of dimensions : at the end of an hour's watching, no further alterations. No pigmentation here, though present in other discs, near which were apparently undergoing transitions to free pigmented organisms, both spherules and crescents. Aspect not uncommon. From Case NO. 7, just after admission and during decline of fever (e.t. 100° F.), under the influence of quinine (20 grains).

FIG. 2. Two red corpuscles from the same patient, on the following day, e.t. 97·4° F. : quinine continued. The plasmic body in the *upper disc* contains a dark granule, active, and, with its containing space, changing form and site. The *lower disc* presents four pale specks on its surface ; similar plasmic specks were seen in the free state around : a notched appearance of the edge of the disc not rare, is also shown.

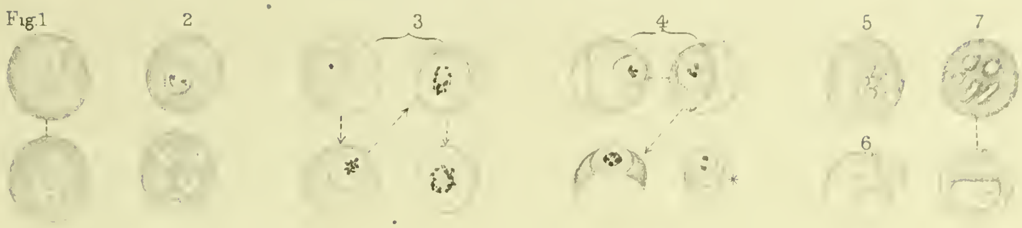
FIG. 3. A Series of four blood-discs from the same patient, selected as indicating one mode in which pigmented *Spherules* may be formed ; namely, by the gradual growth of a pigmented plasmic body, first attached near border of disc, and in the course of a few hours invading its whole area ; blanching and some contraction in diameter of the disc consentaneously occurring. Free dotted, plasmic specks in the fluid medium around. Date—the second day after arrest of fever by quinine, with continued exhibition of the drug : the spleen enlarged and tender. For similar disc-aspects, see also below series B, *b*, spherules bare, fig. 6 *, from another patient.

FIG. 4. A series of four blood-discs, from the same patient and at similar date ; indicating one way, at least, in which pigmented *Crescents* may be formed ; namely, by attachment and growth of a pigmented body, with consentaneous collection around it of the coloured plasma of the disc ; whereby, at the opposite pole of the disc, the stroma becomes blanched and finally forms the fringe of the crescent. * Is a side view, showing the turgidity and rather lessened diameter of the implicated blood-corpuscle ; see also Series B, *a*, Crescents, Figure 3, below. Crescentic organisms being persistent, continue to grow in dimensions after their formation as above.

FIG. 5. A red blood-disc, with pale, active *coccus*-like bodies on its surface, which on watching for some hours, did not show further development : the cor-

# BLOOD-ORGANISMS OF AGUE

## A. Intra-disc Forms and Aspects




## B. Free Forms *a.* The Crescentic


## *b.* The Spheroidal Bare

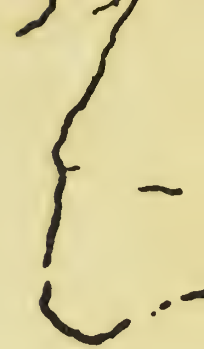

## Flagellated Organisms


## Melanæmia


**Bombay,**
*July—September 1887.*

Lithographed at the Survey of India Offices, Calcutta. January 1888

H. V. C. ad nat. del.



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# SCIENTIFIC MEMOIRS

BY

# MEDICAL OFFICERS OF THE ARMY OF INDIA.

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## PART III.

## 1887.



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CALCUTTA:
PRINTED BY THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.
1888.

Price 3 rupees.